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HOMAGE TO SANTA ROSALIA
or
WHY ARE THERE SO MANY KINDS OF ANIMALS?*

G. E. HUTCHINSON
Department of Zoology, Yale University, New Haven, Connecticut

When you did me the honor of asking me to fill your presidential chair, I accepted perhaps without duly considering the duties of the president of a society, founded largely to further the study of evolution, at the close of the year that marks the centenary of Darwin and Wallace's initial presentation of the theory of natural selection. It seemed to me that most of the significant aspects of modern evolutionary theory have come either from geneticists, or from those heroic museum workers who suffering through years of neglect, were able to establish about 20 years ago what has come to be called the "new systematics." You had, however, chosen an ecologist as your president and one of that school at times supposed to study the environment without any relation to the organism.

A few months later I happened to be in Sicily. An early interest in zoogeography and in aquatic insects led me to attempt to collect near Palermo, certain species of water-bugs, of the genus Corixa, described a century ago by Fieber and supposed to occur in the region, but never fully reinvestigated. It is hard to find suitable localities in so highly cultivated a landscape as the Concha d'Oro. Fortunately, I was driven up Monte Pellegrino, the hill that rises to the west of the city, to admire the view. A little below the summit, a church with a simple baroque facade stands in front of a cave in the limestone of the hill. Here in the 16th century a stalactite encrusted skeleton associated with a cross and twelve beads was discovered. Of this skeleton nothing is certainly known save that it is that of Santa Rosalia, a saint of whom little is reliably reported save that she seems to have lived in the 12th century, that her skeleton was found in this cave, and that she has been the chief patroness of Palermo ever since. Other limestone caverns on Monte Pellegrino had yielded bones of extinct pleistocene Equus, and on the walls of one of the rock shelters at the bottom of the hill there are beautiful Gravettian engravings. Moreover, a small relic of the saint that I saw in the treasury of the Cathedral of Monreale has a venerable and

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petrified appearance, as might be expected. Nothing in her history being known to the contrary, perhaps for the moment we may take Santa Rosalia as the patroness of evolutionary studies, for just below the sanctuary, fed no doubt by the water that percolates through the limestone cracks of the mountain, and which formed the sacred cave, lies a small artificial pond, and when I could get to the pond a few weeks later, I got from it a hint of what I was looking for.

Vast numbers of Corixidae were living in the water. At first I was rather disappointed because every specimen of the larger of the two species present was a female, and so lacking in most critical diagnostic features, while both sexes of the second slightly smaller species were present in about equal number. Examination of the material at leisure, and of the relevant literature, has convinced me that the two species are the common European *C. punctata* and *C. affinis*, and that the peculiar Mediterranean species are illusionary. The larger *C. punctata* was clearly at the end of its breeding season, the smaller *C. affinis* was probably just beginning to breed. This is the sort of observation that any naturalist can and does make all the time. It was not until I asked myself why the larger species should breed first, and then the more general question as to why there should be two and not 20 or 200 species of the genus in the pond, that ideas suitable to present to you began to emerge. These ideas finally prompted the very general question as to why there are such an enormous number of animal species.

There are at the present time supposed to be (Muller and Campbell, 1954; Hyman, 1955) about one million described species of animals. Of these about three-quarters are insects, of which a quite disproportionately large number are members of a single order, the Coleoptera.[1] The marine fauna although it has at its disposal a much greater area than has the terrestrial, lacks this astonishing diversity (Thorson, 1958). If the insects are excluded, it would seem to be more diverse. The proper answer to my initial question would be to develop a theory at least predicting an order of magnitude for the number of species of 10^6 rather than 10^5 or 10^4. This I certainly cannot do. At most it is merely possible to point out some of the factors which would have to be considered if such a theory was ever to be constructed.

Before developing my ideas I should like to say that I subscribe to the view that the process of natural selection, coupled with isolation and later mutual invasion of ranges leads to the evolution of sympatric species, which at equilibrium occupy distinct niches, according to the Volterra-Gause principle. The empirical reasons for adopting this view and the correlative view that the boundaries of realized niches are set by competition are mainly indirect. So far as niches may be defined in terms of food, the subject has been carefully considered by Lack (1954). In general all the indirect evi-

[1]There is a story, possibly apocryphal, of the distinguished British biologist, J. B. S. Haldane, who found himself in the company of a group of theologians. On being asked what one could conclude as to the nature of the Creator from a study of his creation, Haldane is said to have answered, "An inordinate fondness for beetles."

dence is in accord with the view, which has the advantage of confirming theoretical expectation. Most of the opinions that have been held to the contrary appear to be due to misunderstandings and to loose formulation of the problem (Hutchinson, 1958).

In any study of evolutionary ecology, food relations appear as one of the most important aspects of the system of animate nature. There is quite obviously much more to living communities than the raw dictum "eat or be eaten," but in order to understand the higher intricacies of any ecological system, it is most easy to start from this crudely simple point of view.

FOOD CHAINS

Animal ecologists frequently think in terms of food chains, of the form *individuals of species S_1 are eaten by those of S_2, of S_2 by S_3, of S_3 by S_4*, etc. In such a food chain S_1 will ordinarily be some holophylic organism or material derived from such organisms. The simplest case is that in which we have a true *predator chain* in Odum's (1953) convenient terminology, in which the lowest link is a green plant, the next a herbivorous animal, the next a primary carnivore, the next a secondary carnivore, etc. A specially important type of predator chain may be designated Eltonian, because in recent years C. S. Elton (1927) has emphasized its widespread significance, in which the predator at each level is larger and rarer than its prey. This phenomenon was recognized much earlier, notably by A. R. Wallace in his contribution to the 1858 communication to the Linnean Society of London.

In such a system we can make a theoretical guess of the order of magnitude of the diversity that a single food chain can introduce into a community. If we assume that in general 20 per cent of the energy passing through one link can enter the next link in the chain, which is overgenerous (cf. Lindeman, 1942; Slobodkin in an unpublished study finds 13 per cent as a reasonable upper limit) and if we suppose that each predator has twice the mass, (or 1.26 the linear dimensions) of its prey, which is a very low estimate of the size difference between links, the fifth animal link will have a population of one ten thousandth (10^{-4}) of the first, and the fiftieth animal link, if there was one, a population of 10^{-49} the size of the first. Five animal links are certainly possible, a few fairly clear cut cases having been in fact recorded. If, however, we wanted 50 links, starting with a protozoan or rotifer feeding on algae with a density of 10^6 cells per ml, we should need a volume of 10^{26} cubic kilometers to accommodate on an average one specimen of the ultimate predator, and this is vastly greater than the volume of the world ocean. Clearly the Eltonian food-chain of itself cannot give any great diversity, and the same is almost certainly true of the other types of food chain, based on detritus feeding or on parasitism.

Natural selection

Before proceeding to a further consideration of diversity, it is, however, desirable to consider the kinds of selective force that may operate on a food chain, for this may limit the possible diversity.

It is reasonably certain that natural selection will tend to maintain the efficiency of transfer from one level to another at a maximum. Any increase in the predatory efficiency of the n^{th} link of a simple food chain will however always increase the possibility of the extermination of the $(n-1)^{th}$ link. If this occurs either the species constituting the n^{th} link must adapt itself to eating the $(n-2)^{th}$ link or itself become extinct. This process will in fact tend to shortening of food chains. A lengthening can presumably occur most simply by the development of a new terminal carnivore link, as its niche is by definition previously empty. In most cases this is not likely to be easy. The evolution of the whale-bone whales, which at least in the case of *Balaenoptera borealis*, can feed largely on copepods and so rank on occasions as primary carnivores (Bigelow, 1926), presumably constitutes the most dramatic example of the shortening of a food chain. Mechanical considerations would have prevented the evolution of a larger rarer predator, until man developed essentially non-Eltonian methods of hunting whales.

Effect of size

A second important limitation of the length of a food chain is due to the fact that ordinarily animals change their size during free life. If the terminal member of a chain were a fish that grew from say one cm to 150 cms in the course of an ordinary life, this size change would set a limit by competition to the possible number of otherwise conceivable links in the 1-150 cm range. At least in fishes this type of process (metaphoetesis) may involve the smaller specimens belonging to links below the larger and the chain length is thus lengthened, though under strong limitations, by cannibalism.

We may next enquire into what determines the number of food chains in a community. In part the answer is clear, though if we cease to be zoologists and become biologists, the answer begs the question. Within certain limits, the number of kinds of primary producers is certainly involved, because many herbivorous animals are somewhat eclectic in their tastes and many more limited by their size or by such structural adaptations for feeding that they have been able to develop.

Effects of terrestrial plants

The extraordinary diversity of the terrestrial fauna, which is much greater than that of the marine fauna, is clearly due largely to the diversity provided by terrestrial plants. This diversity is actually two-fold. Firstly, since terrestrial plants compete for light, they have tended to evolve into structures growing into a gaseous medium of negligible buoyancy. This has led to the formation of specialized supporting, photosynthetic, and reproductive structures which inevitably differ in chemical and physical properties. The ancient Danes and Irish are supposed to have eaten elm-bark, and sometimes sawdust, in periods of stress, has been hydrolyzed to produce edible carbohydrate; but usually man, the most omnivorous of all animals, has avoided

almost all parts of trees except fruits as sources of food, though various individual species of animals can deal with practically every tissue of many arboreal species. A major source of terrestrial diversity was thus introduced by the evolution of almost 200,000 species of flowering plants, and the three quarters of a million insects supposedly known today are in part a product of that diversity. But of itself merely providing five or ten kinds of food of different consistencies and compositions does not get us much further than the five or ten links of an Eltonian pyramid. On the whole the problem still remains, but in the new form: why are there so many kinds of plants? As a zoologist I do not want to attack that question directly, I want to stick with animals, but also to get the answer. Since, however, the plants are part of the general system of communities, any sufficiently abstract properties of such communities are likely to be relevant to plants as well as to herbivores and carnivores. It is, therefore, by being somewhat abstract, though with concrete zoological details as examples, that I intend to proceed.

INTERRELATIONS OF FOOD CHAINS

Biological communities do not consist of independent food chains, but of food webs, of such a kind that an individual at any level (corresponding to a link in a single chain) can use some but not all of the food provided by species in the levels below it.

It has long been realized that the presence of two species at any level, either of which can be eaten by a predator at a level above, but which may differ in palatability, ease of capture or seasonal and local abundance, may provide alternative foods for the predator. The predator, therefore, will neither become extinct itself nor exterminate its usual prey, when for any reason, not dependent on prey-predator relationships, the usual prey happens to be abnormally scarce. This aspect of complicated food webs has been stressed by many ecologists, of whom the Chicago school as represented by Allee, Emerson, Park, Park and Schmidt (1949), Odum (1953) and Elton (1958), may in particular be mentioned. Recently MacArthur (1955) using an ingenious but simple application of information theory has generalized the points of view of earlier workers by providing a formal proof of the increase in stability of a community as the number of links in its food web increases.

MacArthur concludes that in the evolution of a natural community two partly antagonistic processes are occurring. More efficient species will replace less efficient species, but more stable communities will outlast less stable communities. In the process of community formation, the entry of a new species may involve one of three possibilities. It may completely displace an old species. This of itself does not necessarily change the stability, though it may do so if the new species inherently has a more stable population (cf. Slobodkin, 1956) than the old. Secondly, it may occupy an unfilled niche, which may, by providing new partially independent links, increase stability. Thirdly, it may partition a niche with a pre-existing species. Elton (1958) in a fascinating work largely devoted to the fate of species accidentally or purposefully introduced by man, concludes that in very

diverse communities such introductions are difficult. Early in the history of a community we may suppose many niches will be empty and invasion will proceed easily; as the community becomes more diversified, the process will be progressively more difficult. Sometimes an extremely successful invader may oust a species but add little or nothing to stability, at other times the invader by some specialization will be able to compete successfully for the marginal parts of a niche. In all cases it is probable that invasion is most likely when one or more species happen to be fluctuating and are underrepresented at a given moment. As the communities build up, these opportunities will get progressively rarer. In this way a complex community containing some highly specialized species is constructed asymptotically.

Modern ecological theory therefore appears to answer our initial question at least partially by saying that there is a great diversity of organisms because communities of many diversified organisms are better able to persist than are communities of fewer less diversified organisms. Even though the entry of an invader which takes over part of a niche will lead to the reduction in the *average* population of the species originally present, it will also lead to an increase in stability reducing the risk of the original population being at times underrepresented to a dangerous degree. In this way loss of some niche space may be compensated by reduction in the amplitude of fluctuations in a way that can be advantageous to both species. The process however appears likely to be asymptotic and we have now to consider what sets the asymptote, or in simpler words why are there not more different kinds of animals?

LIMITATION OF DIVERSITY

It is first obvious that the processes of evolution of communities must be under various sorts of external control, and that in some cases such control limits the possible diversity. Several investigators, notably Odum (1953) and MacArthur (1955), have pointed out that the more or less cyclical oscillations observed in arctic and boreal fauna may be due in part to the communities not being sufficiently complex to damp out oscillations. It is certain that the fauna of any such region is qualitatively poorer than that of warm temperate and tropical areas of comparable effective precipitation. It is probably considered to be intuitively obvious that this should be so, but on analysis the obviousness tends to disappear. If we can have one or two species of a large family adapted to the rigors of Arctic existence, why can we not have more? It is reasonable to suppose that the total biomass may be involved. If the fundamental productivity of an area is limited by a short growing season to such a degree that the total biomass is less than under more favorable conditions, then the rarer species in a community may be so rare that they do not exist. It is also probable that certain absolute limitations on growth-forms of plants, such as those that make the development of forest impossible above a certain latitude, may in so acting, severely limit the number of niches. Dr. Robert MacArthur points out that the development of high tropical rain forest increases the bird fauna more than that of mam-

mals, and Thorson (1957) likewise has shown that the so-called infauna show no increase of species toward the tropics while the marine epifauna becomes more diversified. The importance of this aspect of the plant or animal substratum, which depends largely on the length of the growing season and other aspects of productivity is related to that of the environmental mosaic discussed later.

We may also inquire, but at present cannot obtain any likely answer, whether the arctic fauna is not itself too young to have achieved its maximum diversity. Finally, the continual occurrence of catastrophes, as Wynne-Edwards (1952) has emphasized, may keep the arctic terrestrial community in a state of perennial though stunted youth.

Closely related to the problems of environmental rigor and stability, is the question of the absolute size of the habitat that can be colonized. Over much of western Europe there are three common species of small voles, namely *Microtus arvalis*, *M. agrestis* and *Clethrionomys glareolus*. These are sympatric but with somewhat different ecological preferences.

In the smaller islands off Britain and in the English channel, there is only one case of two species co-occurring on an island, namely *M. agrestis* and Clethrionomys on the island of Mull in the Inner Hebrides (Barrett-Hamilton and Hinton, 1911–1921). On the Orkneys the single species is *M. orcadensis*, which in morphology and cytology is a well-differentiated ally of *M. arvalis*; a comparable animal (*M. sarnius*) occurs on Guernsey. On most of the Scottish Islands only subspecies of *M. agrestis* occur, but on Mull and Raasay, on the Welsh island of Skomer, as well as on Jersey, races of Clethrionomys of somewhat uncertain status are found. No voles have reached Ireland, presumably for paleogeographic reasons, but they are also absent from a number of small islands, notably Alderney and Sark. The last named island must have been as well placed as Guernsey to receive *Microtus arvalis*. Still stranger is the fact that although it could not have got to the Orkneys without entering the mainland of Britain, no vole of the *arvalis* type now occurs in the latter country. Cases of this sort may be perhaps explained by the lack of favorable refuges in randomly distributed very unfavorable seasons or under special kinds of competition. This explanation is very reasonable as an explanation of the lack of Microtus on Sark, where it may have had difficulty in competing with *Rattus rattus* in a small area. It would be stretching one's credulity to suppose that the area of Great Britain is too small to permit the existence of two sympatric species of Microtus, but no other explanation seems to have been proposed.

It is a matter of considerable interest that Lack (1942) studying the populations of birds on some of these small British islands concluded that such populations are often unstable, and that the few species present often occupied larger niches than on the mainland in the presence of competitors. Such faunas provide examples of communities held at an early stage in development because there is not enough space for the evolution of a fuller and more stable community.

NICHE REQUIREMENTS

The various evolutionary tendencies, notably metaphoetesis, which operate on single food chains must operate equally on the food-web, but we also have a new, if comparable, problem as to how much difference between two species at the same level is needed to prevent them from occupying the same niche. Where metric characters are involved we can gain some insight into this extremely important problem by the study of what Brown and Wilson (1956) have called *character displacement* or the divergence shown when two partly allopatric species of comparable niche requirements become sympatric in part of their range.

I have collected together a number of cases of mammals and birds which appear to exhibit the phenomenon (table 1). These cases involve metric characters related to the trophic apparatus, the length of the culmen in birds and of the skull in mammals appearing to provide appropriate measures. Where the species co-occur, the ratio of the larger to the small form varies from 1.1 to 1.4, the mean ratio being 1.28 or roughly 1.3. This latter figure may tentatively be used as an indication of the kind of difference necessary to permit two species to co-occur in different niches but at the same level of a food-web. In the case of the aquatic insects with which I began my address, we have over most of Europe three very closely allied species of Corixa, the largest *punctata*, being about 116 per cent longer than the middle sized species *macrocephala*, and 146 per cent longer than the small species *affinis*. In northwestern Europe there is a fourth species, *C. dentipes*, as large as *C. punctata* and very similar in appearance. A single observation (Brown, 1948) suggests that this is what I have elsewhere (Hutchinson, 1951) termed a fugitive species, maintaining itself in the face of competition mainly on account of greater mobility. According to Macan (1954) while both *affinis* and *macrocephala* may occur with *punctata* they never are found with each other, so that all three species never occur together. In the eastern part of the range, *macrocephala* drops out, and *punctata* appears to have a discontinuous distribution, being recorded as far east as Simla, but not in southern Persia or Kashmir, where *affinis* occurs. In these eastern localities, where it occurs by itself, *affinis* is larger and darker than in the west, and superficially looks like *macrocephala* (Hutchinson, 1940).

This case is very interesting because it looks as though character displacement is occurring, but that the size differences between the three species are just not great enough to allow them all to co-occur. Other characters than size are in fact clearly involved in the separation, *macrocephala* preferring deeper water than *affinis* and the latter being more tolerant of brackish conditions. It is also interesting because it calls attention to a marked difference that must occur between hemimetabolous insects with annual life cycles involving relatively long growth periods, and birds or mammals in which the period of growth in length is short and of a very special nature compared with the total life span. In the latter, niche separation may be possible merely through genetic size differences, while in a pair of ani-

TABLE 1

Mean character displacement in measurable trophic structures in mammals (skull) and birds (culmen); data for Mustela from Miller (1912); Apodemus from Cranbrook (1957); Sitta from Brown and Wilson (1956) after Vaurie; Galapagos finches from Lack (1947)

	Locality and measurement when sympatric	Locality and measurement when allopatric	Ratio when sympatric
Mustela nivalis	Britain; skull ♂ 39.3 ♀ 33.6 mm.	(*boccamela*) S. France, Italy ♂ 42.9 ♀ 34.7 mm.	
		(*iberica*) Spain, Portugal ♂ 40.4 ♀ 36.0	♂ 100:128 ♀ 100:134
M. erminea	Britain; " ♂ 50.4 ♀ 45.0	(*bibernica*) Ireland ♂ 46.0 ♀ 41.9	
Apodemus sylvaticus	Britain; " 24.8	unnamed races on Channel Islands 25.6–26.7	100:109
A. flavicollis	Britain; " 27.0		
Sitta tephronota	Iran; culmen 29.0	races east of overlap 25.5	100:124
S. neumayer	Iran; " 23.5	races west of overlap 26.0	
Geospiza fortis	Indefatigable Isl.; culmen 12.0	Daphne Isl. 10.5	100:143
G. fuliginosa	Indefatigable Isl.; " 8.4	Crossman Isl. 9.3	
Camarhynchus parvulus	James Isl.; " 7.0	N. Albemarle Isl. 7.0	James 100:140:180
	Indefatigable Isl.; " 7.5	Chatham Isl. 8.0	100:129
C. psittacula	S. Albemarle Isl.; " 7.3	Abington Isl. 10.1	Indefatigable 100:128:162
	James Isl.; " 9.8	Bindloe Isl. 10.5	100:127
	Indefatigable Isl.; " 9.6		
C. pallidus	S. Albemarle Isl.; " 8.5		
	James Isl.; " 12.6	N. Albemarle Isl. 11.7	S. Albemarle 100:116:153
	Indefatigable Isl.; " 12.1	Chatham Isl. 10.8	100:132
	S. Albemarle Isl.; " 11.2		
			Mean ratio 100:128

mals like *C. punctata* and *C. affinis* we need not only a size difference but a seasonal one in reproduction; this is likely to be a rather complicated matter. For the larger of two species always to be larger, it must never breed later than the smaller one. I do not doubt that this is what was happening in the pond on Monte Pellegrino, but have no idea how the difference is achieved.

I want to emphasize the complexity of the adaptation necessary on the part of two species inhabiting adjacent niches in a given biotope, as it probably underlies a phenomenon which to some has appeared rather puzzling. MacArthur (1957) has shown that in a sufficiently large bird fauna, in a uniform undisturbed habitat, areas occupied by the different species appear to correspond to the random non-overlapping fractionation of a plane or volume. Kohn (1959) has found the same thing for the cone-shells (Conus) on the Hawaiian reefs. This type of arrangement almost certainly implies such individual and unpredictable complexities in the determination of the niche boundaries, and so of the actual areas colonized, that in any overall view, the process would appear random. It is fairly obvious that in different types of community the divisibility of niches will differ and so the degree of diversity that can be achieved. The fine details of the process have not been adequately investigated, though many data must already exist that could be organized to throw light on the problem.

MOSAIC NATURE OF THE ENVIRONMENT

A final aspect of the limitation of possible diversity, and one that perhaps is of greatest importance, concerns what may be called the mosaic nature of the environment. Except perhaps in open water when only uniform quasi-horizontal surfaces are considered, every area colonized by organisms has some local diversity. The significance of such local diversity depends very largely on the size of the organisms under consideration. In another paper MacArthur and I (Hutchinson and MacArthur, 1959) have attempted a theoretical formulation of this property of living communities and have pointed out that even if we consider only the herbivorous level or only one of the carnivorous levels, there are likely, above a certain lower limit of size, to be more species of small or medium sized organisms than of large organisms. It is difficult to go much beyond crude qualitative impressions in testing this hypothesis, but we find that for mammal faunas, which contain such diverse organisms that they may well be regarded as models of whole faunas, there is a definite hint of the kind of theoretical distribution that we deduce. In qualitative terms the phenomenon can be exemplified by any of the larger species of ungulates which may require a number of different kinds of terrain within their home ranges, any one of which types of terrain might be the habitat of some small species. Most of the genera or even subfamilies of very large terrestrial animals contain only one or two sympatric species. In this connection I cannot refrain from pointing out the immense scientific importance of obtaining a really full insight into the ecology of the large mammals of Africa while they can still be studied under natural conditions. It is

indeed quite possible that the results of studies on these wonderful animals would in long-range though purely practical terms pay for the establishment of greater reservations and National Parks than at present exist.

In the passerine birds the occurrence of five or six closely related sympatric species is a commonplace. In the mammal fauna of western Europe no genus appears to contain more than four strictly sympatric species. In Britain this number is not reached even by Mustela with three species, on the adjacent parts of the continent there may be three sympatric shrews of the genus Crocidura and in parts of Holland three of Microtus. In the same general region there are genera of insects containing hundreds of species, as in Athela in the Coleoptera and Dasyhelea in the Diptera Nematocera. The same phenomenon will be encountered whenever any well-studied fauna is considered. Irrespective of their position in a food chain, small size, by permitting animals to become specialized to the conditions offered by small diversified elements of the environmental mosaic, clearly makes possible a degree of diversity quite unknown among groups of larger organisms.

We may, therefore, conclude that the reason why there are so many species of animals is at least partly because a complex trophic organization of a community is more stable than a simple one, but that limits are set by the tendency of food chains to shorten or become blurred, by unfavorable physical factors, by space, by the fineness of possible subdivision of niches, and by those characters of the environmental mosaic which permit a greater diversity of small than of large allied species.

CONCLUDING DISCUSSION

In conclusion I should like to point out three very general aspects of the sort of process I have described. One speculative approach to evolutionary theory arises from some of these conclusions. Just as adaptative evolution by natural selection is less easy in a small population of a species than in a larger one, because the total pool of genetic variability is inevitably less, so it is probable that a group containing many diversified species will be able to seize new evolutionary opportunities more easily than an undiversified group. There will be some limits to this process. Where large size permits the development of a brain capable of much new learnt behavior, the greater plasticity acquired by the individual species will offset the disadvantage of the small number of allied species characteristic of groups of large animals. Early during evolution the main process from the standpoint of community structure was the filling of all the niche space potentially available for producer and decomposer organisms and for herbivorous animals. As the latter, and still more as carnivorous animals began to appear, the persistence of more stable communities would imply splitting of niches previously occupied by single species as the communities became more diverse. As this process continued one would expect the overall rate of evolution to have increased, as the increasing diversity increased the probability of the existence of species preadapted to new and unusual niches. It is reasonable to suppose that strong predation among macroscopic metazoa

did not begin until the late Precambrian, and that the appearance of powerful predators led to the appearance of fossilizable skeletons. This seems the only reasonable hypothesis, of those so far advanced, to account for the relatively sudden appearance of several fossilizable groups in the Lower Cambrian. The process of diversification would, according to this argument, be somewhat autocatakinetic even without the increased stability that it would produce; with the increase in stability it would be still more a self inducing process, but one, as we have seen, with an upper limit. Part of this upper limit is set by the impossibility of having many sympatric allied species of large animals. These however are the animals that can pass from primarily innate to highly modifiable behavior. From an evolutionary point of view, once they have appeared, there is perhaps less need for diversity, though from other points of view, as Elton (1958) has stressed in dealing with human activities, the stability provided by diversity can be valuable even to the most adaptable of all large animals. We may perhaps therefore see in the process of evolution an increase in diversity at an increasing rate till the early Paleozoic, by which time the familiar types of community structure were established. There followed then a long period in which various large and finally large-brained species became dominant, and then a period in which man has been reducing diversity by a rapidly increasing tendency to cause extinction of supposedly unwanted species, often in an indiscriminate manner. Finally we may hope for a limited reversal of this process when man becomes aware of the value of diversity no less in an economic than in an esthetic and scientific sense.

A second and much more metaphysical general point is perhaps worth a moment's discussion. The evolution of biological communities, though each species appears to fend for itself alone, produces integrated aggregates which increase in stability. There is nothing mysterious about this; it follows from mathematical theory and appears to be confirmed to some extent empirically. It is however a phenomenon which also finds analogies in other fields in which a more complex type of behavior, that we intuitively regard as higher, emerges as the result of the interaction of less complex types of behavior, that we call lower. The emergence of love as an antidote to aggression, as Lorenz pictures the process, or the development of cooperation from various forms of more or less inevitable group behavior that Allee (1931) has stressed are examples of this from the more complex types of biological systems.

In the ordinary sense of explanation in science, such phenomena are explicable. The types of holistic philosophy which import *ad hoc* mysteries into science whenever such a situation is met are obviously unnecessary. Yet perhaps we may wonder whether the empirical fact that it is the nature of things for this type of explicable emergence to occur is not something that itself requires an explanation. Many objections can be raised to such a view; a friendly organization of biologists could not occur in a universe in which cooperative behavior was impossible and without your cooperation I could not raise the problem. The question may in fact appear to certain

types of philosophers not to be a real one, though I suspect such philosophers in their desire to demonstrate how often people talk nonsense, may sometimes show less ingenuity than would be desirable in finding some sense in such questions. Even if the answer to such a question were positive, it might not get us very far; to an existentialist, life would have merely provided yet one more problem; students of Whitehead might be made happier, though on the whole the obscurities of that great writer do not seem to generate unhappiness; the religious philosophers would welcome a positive answer but note that it told them nothing that they did not know before; Marxists might merely say, "I told you so." In spite of this I suspect that the question is worth raising, and that it could be phrased so as to provide some sort of real dichotomy between alternatives; I therefore raise it knowing that I cannot, and suspecting that at present others cannot, provide an intellectually satisfying answer.

My third general point is less metaphysical, but not without interest. If I am right that it is easier to have a greater diversity of small than of large organisms, then the evolutionary process in small organisms will differ somewhat from that of large ones. Wherever we have a great array of allied sympatric species there must be an emphasis on very accurate interspecific mating barriers which is unnecessary where virtually no sympatric allies occur. We ourselves are large animals in this sense; it would seem very unlikely that the peculiar lability that seems to exist in man, in which even the direction of normal sexual behavior must be learnt, could have developed to quite the existing extent if species recognition, involving closely related sympatric congeners, had been necessary. Elsewhere (Hutchinson, 1959) I have attempted to show that the difficulties that *Homo sapiens* has to face in this regard may imply various unsuspected processes in human evolutionary selection. But perhaps Santa Rosalia would find at this point that we are speculating too freely, so for the moment, while under her patronage, I will say no more.

ACKNOWLEDGMENTS

Dr. A. Minganti of the University of Palermo enabled me to collect on Monte Pellegrino. Professor B. M. Knox of the Department of Classics of Yale University gave me a rare and elegant word from the Greek to express the blurring of a food chain. Dr. L. B. Slobodkin of the University of Michigan and Dr. R. H. MacArthur of the University of Pennsylvania provided me with their customary kinds of intellectual stimulation. To all these friends I am most grateful.

LITERATURE CITED

Allee, W. C., 1931, Animal aggregations: a study in general sociology. vii, 431 pp. University of Chicago Press, Chicago, Illinois.

Allee, W. C., A. E. Emerson, O. Park, T. Park and K. P. Schmidt, 1949, Principles of animal ecology. xii, 837 pp. W. B. Saunders Co., Philadelphia, Pennsylvania.

Barrett-Hamilton, G. E. H., and M. A. C. Hinton, 1911–1921, A history of British mammals. Vol. 2. 748 pp. Gurney and Jackson, London, England.

Bigelow, H. B., 1926, Plankton of the offshore waters of the Gulf of Maine. Bull. U. S. Bur. Fisheries 40: 1–509.

Brown, E. S., 1958, A contribution towards an ecological survey of the aquatic and semi-aquatic Hemiptera-Heteroptera (water-bugs) of the British Isles etc. Trans. Soc. British Entom. 9: 151–195.

Brown, W. L., and E. O. Wilson, 1956, Character displacement. Systematic Zoology 5: 49–64.

Cranbrook, Lord, 1957, Long-tailed field mice (Apodemus) from the Channel Islands. Proc. Zool. Soc. London 128: 597–600.

Elton, C. S., 1958, The ecology of invasions by animals and plants. 159 pp. Methuen Ltd., London, England.

Hutchinson, G. E., 1951, Copepodology for the ornithologist. Ecology 32: 571–577.

1958, Concluding remarks. Cold Spring Harbor Symp. Quant. Biol. 22: 415–427.

1959, A speculative consideration of certain possible forms of sexual selection in man. Amer. Nat. 93: 81–92.

Hutchinson, G. E., and R. MacArthur, 1959, A theoretical ecological model of size distributions among species of animals. Amer. Nat. 93: 117–126.

Hyman, L. H., 1955, How many species? Systematic Zoology 4: 142–143.

Kohn, A. J., 1959, The ecology of Conus in Hawaii. Ecol. Monogr. (in press).

Lack, D., 1942, Ecological features of the bird faunas of British small islands. J. Animal Ecol. London 11: 9–36.

1947, Darwin's Finches. x, 208 pp. Cambridge University Press, Cambridge, England.

1954, The natural regulation of animal numbers. viii, 347 pp. Clarendon Press, Oxford, England.

Lindeman, R. L., 1942, The trophic-dynamic aspect of ecology. Ecology 23: 399–408.

Macan, T. T., 1954, A contribution to the study of the ecology of Corixidae (Hemipt). J. Animal Ecol. 23: 115–141.

MacArthur, R. H., 1955, Fluctuations of animal populations and a measure of community stability. Ecology 35: 533–536.

1957, On the relative abundance of bird species. Proc. Nat. Acad. Sci. Wash. 43: 293–295.

Miller, G. S., Catalogue of the mammals of Western Europe. xv, 1019 pp. British Museum, London, England.

Muller, S. W., and A. Campbell, 1954, The relative number of living and fossil species of animals. Systematic Zoology 3: 168–170.

Odum, E. P., 1953, Fundamentals of ecology. xii, 387 pp. W. B. Saunders Co., Philadelphia, Pennsylvania, and London, England.

Slobodkin, L. B., 1955, Condition for population equilibrium. Ecology 35: 530–533.

Thorson, G., 1957, Bottom communities. Chap. 17 *in* Treatise on marine ecology and paleoecology. Vol. 1. Geol. Soc. Amer. Memoir 67: 461–534.

Wallace, A. R., 1858, On the tendency of varieties to depart indefinitely from the original type. *In* C. Darwin and A. R. Wallace, On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection. J. Linn. Soc. (Zool.) 3: 45–62.

Wynne-Edwards, V. C., 1952, Zoology of the Baird Expedition (1950). I. The birds observed in central and southeast Baffin Island. Auk 69: 353–391.

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STUDY OF FERTILIZATION AND EMBRYOGENESIS IN CERTAIN ANGIOSPERMS USING LIVING MATERIAL

V. A. PODDUBNAYA-ARNOLDI

Main Botanical Garden, U.S.S.R. Academy of Sciences, Moscow U-276

Recently in vivo studies have made considerable progress (P. Maheshwari, 1950). In the U.S.S.R., K. J. Kostriukova, G. K. Benetskaya, M. V. Tschernoyarov, M. S. Yakovlev, M. D. Yoffe and the writer have used the in vivo method for studying pollen grains, pollen tubes, embryo sacs, endosperm and embryos. This has led to some new and interesting findings.

Macrosporogenesis, various developmental stages of the embryo sac, fertilization and early embryo development have usually been followed on fixed material. Hence, our investigations have been focussed on the in vivo studies of precisely these phases of development. Unsectioned total preparations of live pollen grains, pollen tubes, ovules and seeds have been used.

Since Strasburger (1900) and Shibata (1902) no data from living material have been available on fertilization in angiosperms. Strasburger studied *Monotropa hypopitys*, and Shibata, *Monotropa uniflora*.

Physiological studies of fertilization as well as other embryonic processes necessitate the use of living material. In the orchids *Cypripedium insigne*, *Calanthe Veitchii* and *Dendrobium nobile*, we have studied in vivo not only spermiogenesis, gametogenesis and embryogenesis, but also cytogenesis. In all the above three species the penetration of the pollen tube and fusion of the gametes have frequently been followed up. Our method was extremely simple. The ovules separated from the placenta were placed on a slide in a drop of water or of a ten per cent sugar solution, covered with a coverslip, and examined under water or oil immersion. In this way it was possible to examine promptly numerous diverse objects. The pictures were so distinct and clear that there was no necessity to use the phase contrast or the anoptral microscope.

The orchids provide extremely favorable material for studying embryonic processes in vivo, as their ovules are minute and the integuments thin and transparent. This is a long-known fact which has made the orchids favorite objects of embryological study. A comparison of the patterns of gametophyte and gamete formation, of fertilization and embryogenesis, on living and fixed material, discloses a great similarity. However, in vivo studies reveal some details which escape observation on fixed material, since in vivo the plasma and its inclusions, such as plastids, chondriosomes, fat globules, etc., are better preserved. Moreover, since the in vivo method is simpler it allows more extensive study.

It has thus proved possible to follow the behavior of plastids and chondriosomes at different stages of development of the reproductive organs.

The plastids were found in some cases to be colorless, in others green at various stages of development of anthers and ovules, of microspores and macrospores, of pollen tubes, of male and female gametophytes, as well as of embryos. It was concluded that the plastids play an important role not only in the vegetative organs but in the generative ones as well, being centers of synthesis of various plastic, physiologically active substances and enzymes, such as starch, fat, proteins, ascorbic acid, peroxidase, and others.

In the pollen grains and pollen tubes of the above orchids, the plastids and chondriosomes produce fat. As soon as the pollen tube attains a certain length the inclusions become most abundant in its tip, where the vegetative nucleus is also situated as well as the generative cell and later the sperm cells. An examination of pollen tubes in *Cypripedium insigne*, *Calanthe Veitchii* and *Dendrobium nobile* disclosed some "plugs" which divide the pollen tubes into individual fragments (figure 1 a). These "plugs" apparently prevent the diffusion of nutrient and energetic compounds of the pollen tube throughout its surface. Therefore, they are concentrated in the tip of the pollen tube where its most important elements are located. On the other hand, "plugs" presumably act as a support preventing collapse of the walls of the pollen tube. A detailed examination of these "plugs" showed that they vary in width, that they are composed of the same substance as the membrane of the pollen tube, and that they yield a positive pectin reaction.

Such "plugs" have been noted in the pollen tubes not only of orchids but of other angiosperms as well.

Besides the "plugs," we have observed in some angiosperms a thickening of the membrane of the tip of the pollen tube, a kind of cap which like that of the root apparently plays a protective role in keeping the pollen tube from injury (figure 1 b).

In the orchids only one pollen tube usually enters the ovule, but there may be as many as six. In the above orchids, the pollen tube usually enters the ovule when this contains a ripe embryo sac, but eventually this may happen much earlier when the ovule contains only a tetranucleate embryo sac or even only an archesporal cell.

In the orchid species studied, the sperm are represented by cells which in *Cypripedium insigne* have abundant chondriosomes, plastids, and fat droplets of varying size (figure 2 b). While migrating along the pollen tube, the sperm nuclei alter their shape from rounded to more or less oblong. The more extended they are, the less distinct becomes the surrounding plasma. The sperm nuclei of these orchids contain nucleoli.

Plastids and chrondriosomes have been revealed during macrosporogenesis and development of the embryo sac of the orchids. In some of them (*Cypripedium insigne*) starch is formed in these cellular elements, while in others (*Calanthe Veitchii* and *Dendrobium nobile*) they contain fat.

In *Cypripedium insigne*, the development of the embryo sac follows the bisporic type. The number of nuclei is as a rule less than six (five or six). In Calanthe and Dendrobium the development of the embryo sac is of the

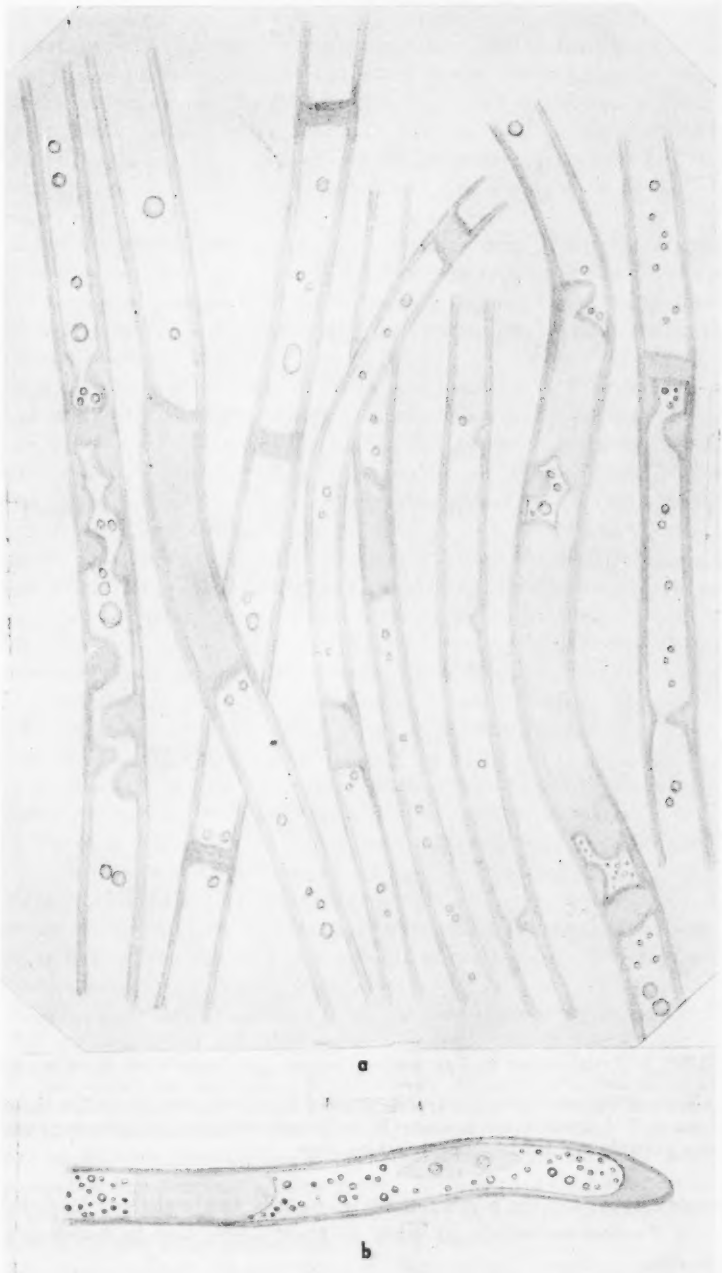

FIGURE 1. "Plugs" in pollen tubes of *Cypripedium insigne* (a) and *Reseda odorata* (b).

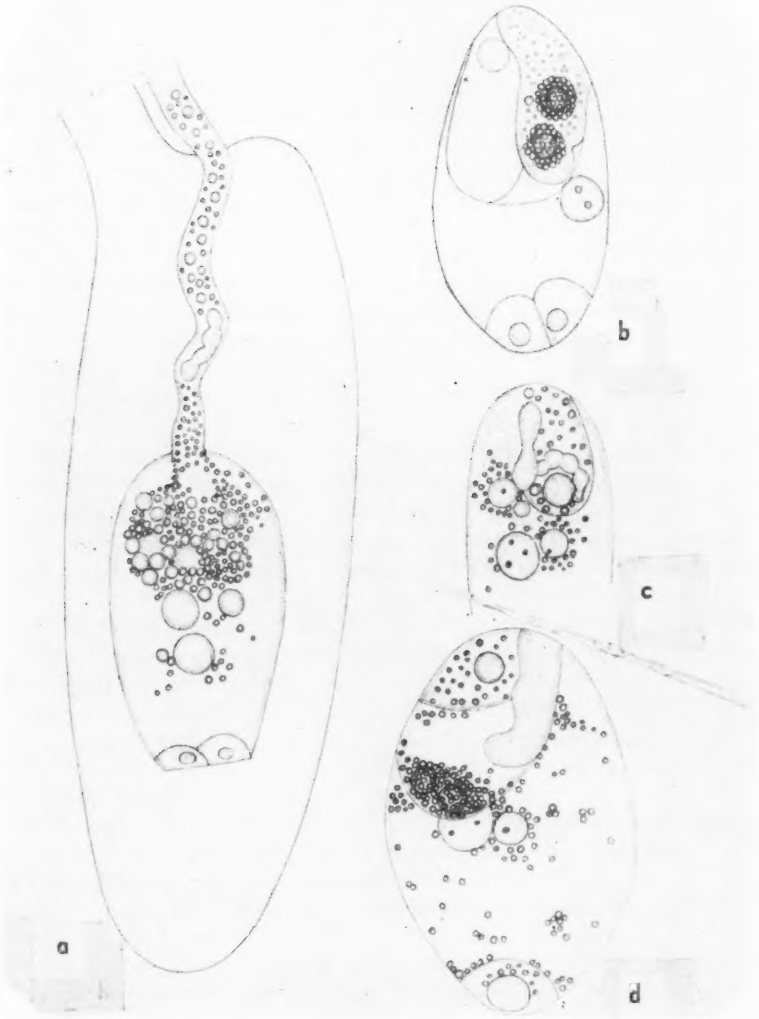

FIGURE 2. Fertilization in *Cypripedium insigne*: a. Penetration of pollen tube into the ovule and embryo sac. b. Penetration of pollen tube into embryo sac. The sperms are cell shaped. c. Sperm approach to the egg-cell and to secondary nucleus of embryo sac. d. Double fertilization. In the plasma of the pollen tube and embryo sac, note plastids and fat droplets of varying size.

monosporic type, and the number of nuclei usually is six or (less frequently) five. In *Dendrobium nobile*, as many as eight nuclei may be found in the embryo sac.

Starch which occurs in the embryo sac of some orchids disappears as fertilization is approached. By the moment of fertilization and soon afterwards,

fat is especially abundant in the embryo sacs and pollen tubes of the orchids (figures 2a–d). This is obviously due to the fact that fat is carried into the embryo sac mostly by the pollen tube, and that it acts not only as a trophic compound but as a carotinoid solvent as well. The carotinoids are claimed to play an important role in the generative processes (P. M. Zhukovsky, J. A. Medvedev, S. I. Lebedev, V. A. Poddubnaya-Arnoldi, N. V. Tzinger and others).

It has frequently been noted that the pollen tube penetrates deeply into the embryo sac, forming loops. Sometimes the embryo sac is filled up with coils of one or even several pollen tubes. In such cases the pollen tube obviously injures all the elements of the embryo sac, so that fertilization is no longer possible.

It is particularly difficult to follow the fusion of the gametes in vivo, since the pollen tube penetrating the embryo sac contains numerous inclusions which mask the nuclei and cells. And yet it often proved possible to follow the fertilization process, especially in Calanthe and Dendrobium where the contents of the pollen tube do not obscure the nuclei and cells as much as in Cypripedium.

In Cypripedium and Dendrobium typical double fertilization was noted (figures 2d and 3a–c) but not in Calanthe (figures 3d–g), as the second sperm cell frequently does not fuse with the polar-antipodal complex of nuclei. These nuclei are very similar in appearance to those of the sperm. The nuclei of the egg-cell and of the sperm which fuses with it are likewise often indistinguishable from one another either in size or in shape. The secondary nuclei of the embryo sac in *Cypripedium insigne* and *Dendrobium nobile* are somewhat larger than those sperm nuclei.

A comparison of the in vivo fertilization picture in *Dendrobium nobile* (figures 3a–c) with that of fixed material shows that in the latter the cells and nuclei have a somewhat lesser size. This difference is obviously due to loss of water caused by fixation.

Once formed the zygote (figure 3g) rapidly increases in size, while in the nucleus two nucleoli are preserved for a long time. A non-differentiated embryo is formed from the zygote of the above orchids by a series of successive divisions.

We have been much concerned in the present study with the cultivation of orchid embryos in an artificial nutrient medium. As to *Dendrobium nobile*, *Calanthe Veitchii*, *Phalaenopsis Schilleriana* and *Cypripedium insigne*, not only seeds containing embryos but also ovules at various stages of development up to fertilization have been cultivated in such media. The ovules of these orchids continued growing and developing on the artifical medium if fertilization had previously taken place. In such cases the first division of the zygote as well as the subsequent stages of embryo development up to the multicellular stage (figures 4a–d) were followed.

As far as we know, no one except N. Maheshwari (1958) has succeeded in observing the initial phases of embryo development of any angiosperm species in tissue cultures. As a rule embryo development was followed on

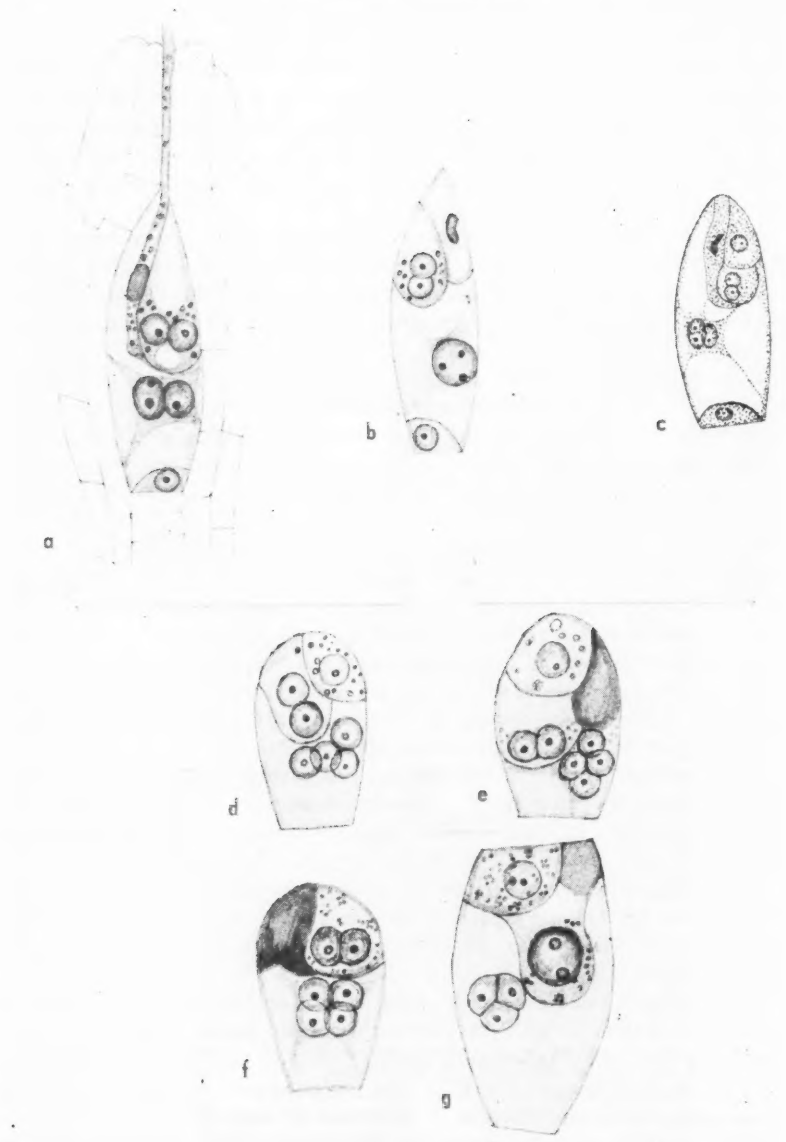

FIGURE 3. Fertilization in *Dendrobium nobile* (a–c) and *Calanthe Veitchii* (d–g). a–b. Double fertilization in live material. c. Double fertilization in fixed material. d–f. Approach of sperms to the nuclei of the egg-cell and of the polar-antipodal complex. g. Zygote. In the plasma of the pollen tube and embryo sac, plastids and fat droplets are to be seen.

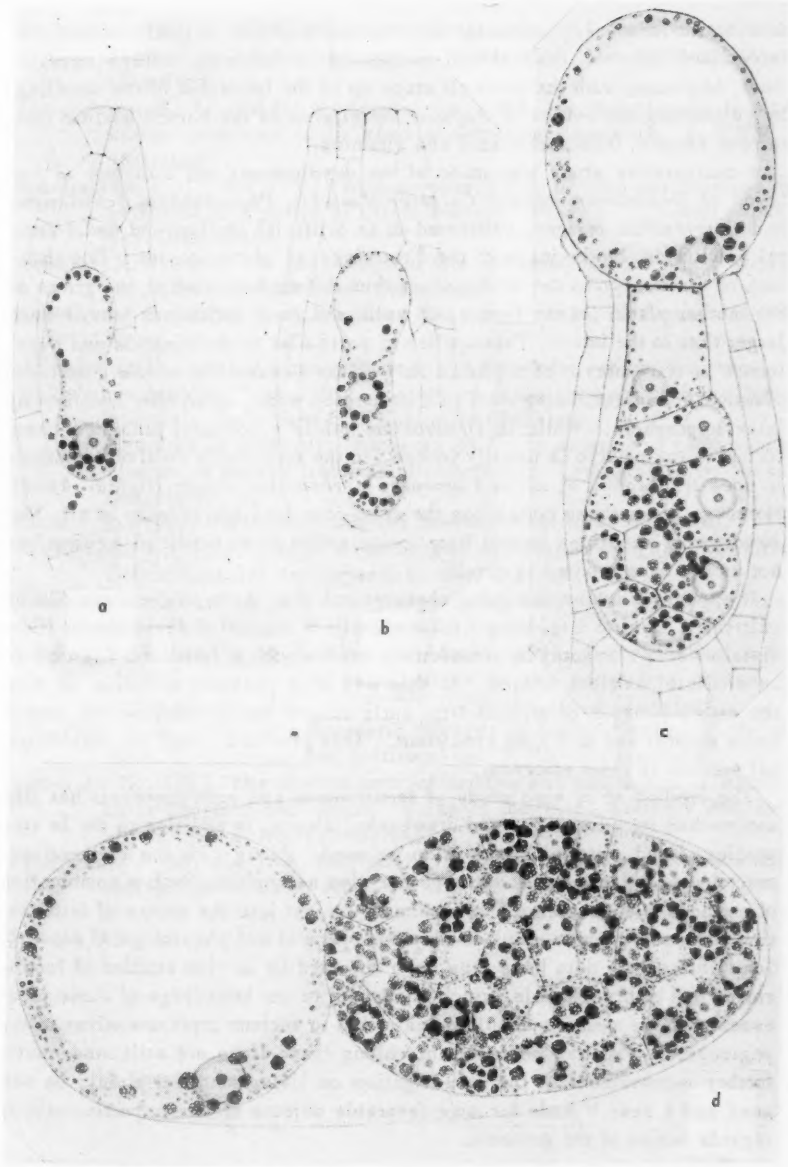

FIGURE 4. Embryogenesis in *Dendrobium nobile* in ovules cultivated on an artificial nutrient medium. a. Zygote. b. Two-celled embryo. c. Ten-celled embryo. d. Multicellular embryo. In the plasma of the embryo sac and of the embryo, plastids can be seen which frequently form aggregates. In figures a–c, besides the embryo sac with an embryo, the cells of the inner integument of the ovule are shown.

artificial media only beginning with the multicellular, slightly or well differentiated embryo. Maheshwari succeeded in following embryo development, beginning with the two-cell stage up to the formation of the seedling, by cultivating the ovules of *Papaver somniferum* in the Nitsch medium containing kinetin, indolacetic acid and vitamins.

A comparative study was made of the development and structure of embryos of *Dendrobium nobile*, *Calanthe Veitchii*, *Phalaenopsis Schilleriana* and *Cyrpipedium insigne*, cultivated in an artificial medium and under natural conditions, beginning with the first cleavage of the zygote. The structure of the embryo in the artificial medium differs from that of one grown on the mother plant. In the former, all cells and their inclusions proved much larger than in the latter. This applies in particular to the plastids and haustorium of the embryo, exemplified as well by *Dendrobium nobile*. Here the plastids are larger, being as a rule connected with one another and forming large aggregates. While in *Dendrobium nobile* a powerful branched haustorium of the embryo is usually formed, in the artificially cultivated embryo it does not branch at all and assumes a vesicular shape (figures 4c-d). Moreover, under these conditions the seed-coat does not develop at all. The structure of seedlings formed from unripe seeds in an artificial medium has not as yet been studied in detail.

It appears from the foregoing observations that the orchid embryo can be cultivated in vitro beginning with the earliest stages of development (first division of the zygote) by transferring ovules with a fertilized egg-cell to an artificial nutrient medium. In this way it is possible to follow in vivo the embryogenesis of orchids from early stages and to compare its course under natural and artificial conditions. This provides a tool for physiological studies in plant embryos.

The method of in vivo study of fertilization and embryogenesis has like any method its advantages and drawbacks. Hence, in addition to the in vivo studies, fixed material should also be used. Along with the conventional procedures, histochemical methods are also advisable. Such a combination of methods will provide a more profound insight into the nature of fertilization and embryogenesis both in its morphological and physiological aspects. Some interesting data have thus been obtained by in vivo studies of fertilization and embryogenesis, which contribute to our knowledge of these processes. These methods should be extended to various representatives of the angiosperms. As the achievements along these lines are still inadequate, further improvement of the investigation on living material should be pursued and a search made for more favorable objects of study, particularly as regards fusion of the gametes.

LITERATURE CITED

Benetskaya, G. K., 1939, In vitro observations on male gametes in pollen tubes of *Asclepias cornuti, Vinca major* and *Vinca minor*. Botanich. J. USSR 3 (Russian).

Kostriukova, K. J., 1939, In vitro observations on the formation of male gametes in *Lilium martagon L.* Dokl. Akad. Sci. USSR 22, No. 7 (Russian).

Kostriukova, K. J., and G. K. Benetskaya, 1939, Spermatogenesis in *Narcissus poeticus L.* in vitro observation. Botanich. J. USSR 3 (Russian).

Kostriukova, K. J., and M. V. Tschernoyarov, 1938, In vitro observations on germination of pollen of *Clivia miniata.* Akad. A. V. Fomin memori Vol. (Ukrainian).

Lebedev, S. I., 1958, The physiological role of carotine in the plant. Acad. Sci. Ukrainian SSR Press (Russian).

Maheshwari, N., 1958, In vitro culture of excised ovules of *Papaver somniferum.* Science 127.

Maheshwari, P., 1950, An introduction to the embryology of angiosperms.

Poddubnaya-Arnoldi, V. A., 1952, A study of angiosperm embryos on living material. Bull. Main Bot. Garden 14 (Russian).

1956, Studies in embryonic processes on living material in some angiosperms. *In* Modern problems of embryology. Leningrad Univ. Press (Russian).

Shibata, L., 1902, Doppelfruchtung bei *Monotropa uniflora.* Flora 90.

Strasburger, E., 1900, Einige Bemerkungen zur Frage nach der doppelten Befruchtung bei Angiospermen. Bot. Ztg. 58.

Tschernoyarov, M. V., 1952, On greenness of developing embryos. Isv. Akad. Sci. USSR 5, No. 7 (Russian).

Tzinger, N. V., and V. A. Poddubnaya-Arnoldi, 1956, A contribution to the physiological role of carotinoides in the generative organs of higher plants. Dokl. Acad. Sci. USSR 110, No. 1 (Russian).

Yakovlev, M. S., 1951, Morphogenetic features of higher plants. Works of the V. L. Komarov Bot. Institute, Ser. 7, No. 11 (Russian).

Yoffe, M. D., 1957, The development of embryo and endosperm in wheat, horse-chestnut and radish. Works of the V. L. Komarov Bot. Institute, Ser. 7, No. 4 (Russian).

Zhukovksy, P. M., and J. A. Medvedev, 1949, Extraplastid carotinoids and sporogenesis. Dokl. Acad. Sci. USSR 14, No. 1 (Russian).

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DIFFERENCES BETWEEN POPULATIONS IN EMBRYONIC DEVELOPMENTAL RATES

JAMES C. KING*

Department of Zoology, Columbia University, New York, New York

INTRODUCTION

An increasing number of students of population genetics is becoming convinced that the modal phenotype of a cross-breeding population is the result of the random, yet systematic formation of more or less heterozygous genotypes. If, by artificial breeding procedures, we make even a substantial portion of the individual genome homozygous, we obtain an array of phenotypes which differs statistically from that of the randomly breeding population (Wallace and King, 1951; 1952). And yet the characteristics of a randomly breeding population cannot be confected at once merely by bringing genetically heterogeneous material together (Dobzhansky, 1950). When a cross between two different populations is made, the F_1 individuals are usually very uniform with respect to size, viability, fecundity and many other measurable characters; but the F_2 of such a cross often possesses different distributions for these characters than were shown by the F_1 or than persist in either of the populations between which the cross was made (Vetukhiv, 1956). These facts among others have led to the concept of the coadaptation or the integration of the genetic system of every established, cross-breeding population. Similar conclusions have been reached by some animal breeders: that the modal phenotype cannot be maintained if the degree of heterozygosity falls below a certain level (Lerner, 1954).

It has been demonstrated that in Drosophila heterozygotes are less affected during development by environmental influences than are homozygotes (Dobzhansky and Wallace, 1953) and this difference has been attributed to a more effective homeostatic mechanism operating in the heterozygotes. The problem of how developmental processes are held within normal limits, which he prefers to call canalization, has been given very considerable attention by Waddington (1958) but he has serious reservations concerning heterozygosity as the explanation.

But although the data and the hypotheses for explaining them cry out for a developmental approach to populations or for a populational approach to development, no experimental work along these lines has been undertaken. During the past two decades population and developmental genetics have developed with almost complete mutual indifference, the former concerning itself with the means and variances of characters and their relation to gene frequencies; the latter refining its descriptions of normal development, using

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observations on the abnormal to elucidate the normal. Neither the population nor the developmental geneticist can ever come to a full understanding of his data until the two approaches are to some degree synthesized. The different distributions of characters in different populations result from events occurring during development; and normal development is produced by the vast majority of the many diverse genomes which result from the random union of gametes in a cross-fertilizing population. The experiments described in this paper were designed as an attempt to begin such a synthesis.

MATERIALS AND METHODS

Since a great deal of the experimental work in population genetics has been done on species of Drosophila, *D. melanogaster*, because of its rapid development and the ease with which large numbers of its eggs may be obtained, was selected as the experimental animal. Several technical problems had to be solved first. In order to look for embryonic characters which could be scored or measured for statistical treatment, a method had to be devised for preparing considerable numbers of embryos for observation. The conventional methods of fixation and staining used by embryologists are unsatisfactory for this purpose, for their technical complexity makes impossible demands on the investigator's time. It was found that very satisfactory temporary preparations could be made by placing a group of about 50 eggs in a small drop of water in a Bureau of Plant Industry watch glass, adding eight to ten drops of boiling water, removing the water after it had cooled by means of a mouth pipette and replacing it with a one-to-one dilution of commercial Clorox. Fifteen to 20 minutes in the Clorox will remove the chorion; the Clorox may then be removed with the mouth pipette and replaced with Ringer's solution. A tiny drop of a dilute solution of a wetting agent such as Triton X placed in the watch glass before the addition of each liquid will keep the eggs from floating. The entire operation may be carried out under a binocular dissecting microscope using either transmitted or reflected light. A serviceable pipette may be constructed by drawing out the end of an ordinary dropper to a fine tip and giving the end a slight curve. The dropper may be fitted with a piece of flexible tubing, the other end of which is held in the mouth. With a little practice one learns to draw the liquid from the watch glass without sucking up the eggs. A small flask inserted as a trap between the pipette and the mouth prevents accidental imbibing of unpalatable reagents. Whole embryos prepared in this way are quite satisfactory for determining the stage of development, and they may be kept in the Ringer's without further treatment for as much as 36 or even 48 hours.

Morphological differences do exist between embryos of the same stage of development, but the most satisfactory characters for beginning this type of investigation are developmental rates. For measuring such rates, one must have groups of eggs laid at approximately the same time. These may be collected by placing a thin slice of agar on a bottle cap, inserting the cap into

a fresh culture bottle containing 200 to 300 five to ten-day old flies and inverting the bottle. By replacing the cap and the slice of agar every 20 minutes, groups of eggs with mean laying times 20 minutes apart may be obtained. They are most conveniently transferred from the agar with a flattened needle. The agar may be prepared by heating three grams of flake agar in 100 ml. of water until it dissolves and pouring the solution into a 100 ml. graduate to harden. If the graduate is heated with hot water, the agar may be shaken out and sliced with a sharp scalpel. The thinner the slice, the better it will adhere to the bottle cap. Unfortunately, flies will not always produce eggs to order. Egg production can be encouraged by making sure that the flies have been well fed with fresh yeast in the 24 hours before the eggs are wanted, and the agar may be made more attractive as an oviposition site by adding three ml. of a one to two mixture of glacial acetic acid and ethanol to the agar solution just before it is poured into the graduate. Painting the agar slice with a thin yeast suspension just before putting it into the bottle is also sometimes effective.

The measurement of developmental rates requires special statistical procedures. The most useful measurements are the T_{50}, the time after egg laying when 50 per cent of the embryos have reached a given stage, and the standard deviation of the developmental distribution. The best procedure for obtaining these is the probit analysis developed by toxicologists for measuring dose-effect relationships. One makes the assumption that an embryo is susceptible to time as an organism is susceptible to a drug. In a series of successive egg samples, all killed at a time estimated to cover the period during which a given stage will have been reached, the embryos are scored on an all-or-nothing basis with respect to arrival at the stage and a per cent arrived is calculated for each group. These per cents are then plotted on probit paper against time lapsed since mean egg-laying time and a regression curve is obtained. Where this line cuts the fifth probit is the T_{50} and the standard deviation of the distribution is the reciprocal of the slope (1/b). These statistics may be precisely calculated and errors for them obtained (Finney, 1952), but often the data give so nearly a normal distribution that the desired values may be read directly from an eye-fitted line. Such statistics are adequate if one makes repeated runs and then calculates the mean values of the T_{50}'s and the 1/b's. From the standard deviations of these means confidence intervals can be obtained. In the case of the T_{50} for egg hatching, for various reasons it is more efficient to set up a group of eggs in rows on an agar slice and make and record counts of the hatched eggs at precise time intervals. The cumulated percentages are then plotted on probit paper.

RESULTS

Measurements have been made of the T_{50}'s and the standard deviation of the developmental distribution (1/b) for five stages of development on three strains of *Drosophila melanogaster*. One of the strains, Syosset (Sy) (King and Sømme, 1958), is a large randomly breeding population maintained in a

cage since 1953. The other two are inbred strains maintained by brother-sister mating for more than 100 generations. One of these, SySP, stems from Sy; the other, I_1, from a laboratory stock of Oregon R. The five stages selected for measurement are I, extending germ band; II, extended germ band; III, contracting germ band; IV, contracted germ band; and V, hatching. Table 1 gives the means of these measurements in minutes and the standard deviations of the means. In figure 1 the mean T_{50} values and their 95 per cent confidence intervals for stages I to IV are shown graphically.

TABLE 1*

T_{50} and standard deviations of measurements on three strains of *D. melanogaster* at five stages of development.

Strain	Stage	n	T_{50}	$s_{T_{50}}$	1/b	$s_{1/b}$
Sy	I	15	187	1.14	9.6	0.40
	II	9	223	4.24	11.4	0.71
	III	21	463	4.93	14.0	0.78
	IV	21	499	4.49	18.8	1.01
	V	9	1,230	28.1	20.9	1.68
SySP	I	8	180	1.62	9.7	0.60
	II	8	229	1.72	12.4	0.75
	III	9	447	5.08	17.4	1.01
	IV	9	494	7.62	19.9	1.20
	V	7	1,328	30.3	40.4	4.60
I_1	I	8	193	2.35	10.2	0.71
	II	8	233	2.09	10.3	1.55
	III	7	501	14.84	18.1	1.37
	IV	7	541	10.93	24.3	2.19
	V	2	1,193	...	36.	...

*T_{50}, mean time of arrival in minutes; 1/b, mean standard deviation of the developmental distributions; $s_{T_{50}}$, $s_{1/b}$, standard deviations of above means; n, numbers of observations.

It can be seen that for some stages the T_{50}'s of some of the strains differ. At stage I, for example, SySP differs significantly from Sy and from I_1, but the latter two do not differ from each other. At stage II there are no significant differences. At stage III Sy overlaps both SySP and I_1, but the latter two differ. At stage IV Sy is completely contained within SySP, but I_1 overlaps neither. The data for stage V are not shown graphically, but inspection of the table indicates no significant differences between strains.

The confidence intervals of the T_{50}'s are large and a considerable part of their size arises from the fact that the T_{50} appears to vary in a non-random fashion from day to day and week to week. Uncontrolled variables in the laboratory environment evidently change its real value. If, instead of comparing mean T_{50}'s, we work with differences between the T_{50}'s of pairs of strains measured on the same day, we should reduce the effects of these environmental influences.

Table 2 gives the mean ($\bar{d}$) of the differences between paired T_{50}'s, the standard deviation of this mean difference ($s_{\bar{d}}$), the ratio of the former to the

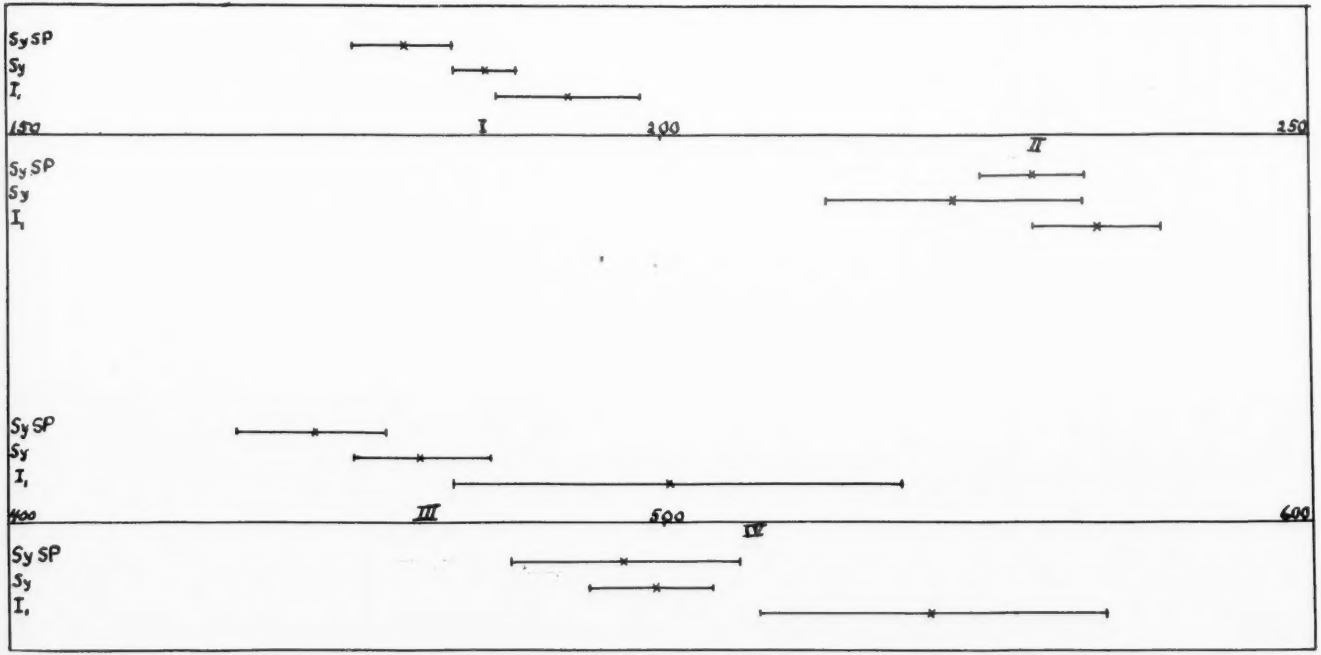

FIGURE 1. Mean T_{50}'s with 95 per cent confidence intervals for three strains of *D. melanogaster* at four stages of embryonic development. Scale in minutes from mean laying time. For definition of stages see text.

latter, which is a t value, and the corresponding probability. This probability figure tells us how likely we would be to observe a mean difference as large or larger than the one observed if there were not real differences between the T_{50}'s. A low probability is evidence of a real difference. A high probability does not mean that the T_{50}'s are identical, but that the difference between them is small with respect to the error of measurement.

Thus at stages I and IV there must be real differences in T_{50} between Sy and I_1; if there are real differences between these strains at stages II and III, they are probably smaller than those at I and IV. For stage V we have too few observations to calculate a precise probability, but the fact that the total of the differences here is two when the T_{50}'s are all over 1,000 strongly suggests that any real difference must be minute. As between Sy and SySP, we have paired observations for stages III, IV, and V only. There appear to be real differences at stages III and V and no difference, or a much smaller one, at stage IV. Figure 2 shows graphically these data from paired observations.

TABLE 2

Mean differences in T_{50}'s, standard deviations of the means, t values and probabilities for paired observations on different strains at different stages of development. Number of paired observations: n.

Pair	Stage	n	$\bar{d}$	$s_{\bar{d}}$	t	P
Sy : I_1	I	4	13.25	3.43	3.870	.02 – .05
	II	4	9.00	6.67	1.349	.20 – .40
	III	6	7.33	9.535	.769	.40 – .60
	IV	6	20.67	4.455	4.640	< .01
	V	2	...	...	...	...
Sy : SySP	III	8	10.125	2.280	4.441	< .01
	IV	8	0.125	3.148	0.040	> .60
	V	3	72.	9.183	7.841	.01 – .02

With respect to 1/b, the standard deviation of the developmental distribution, these measurements have uncovered no significant differences between strains at the first four stages. There does, however, appear to be an increase in this value for all strains as development proceeds. At stage V, however, there is a sharp difference between Sy and SySP, and the meager data available indicate that I_1, with a T_{50} no different from Sy, has a distribution more like that of SySP, which has a distinctly higher T_{50}. The data also indicate, although it is not apparent from the statistics given here, that both SySP and I_1 differ from Sy at stage V in having abnormal distributions with tails much longer than are consistent with that part of the curve lying within one sigma of the mean. This is interpretable as an illustration of the greater variability or poorer canalization or buffering of the homozygotes as compared with heterozygotes.

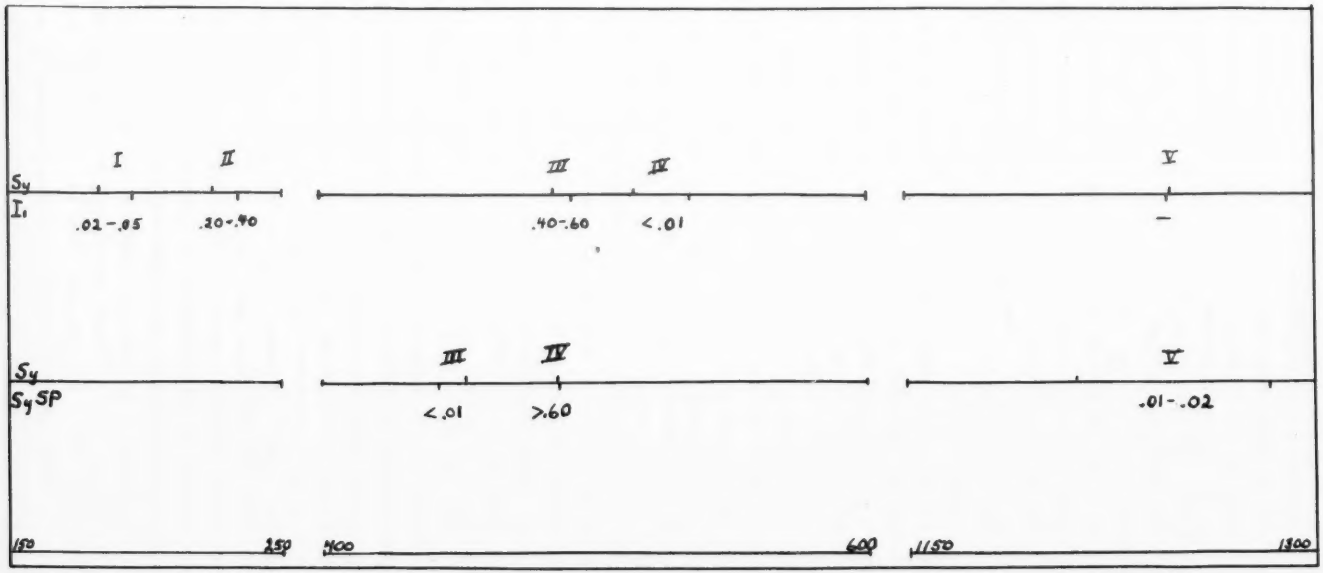

FIGURE 2. Graphic representation of mean differences in T_{50} based on paired observations for two pairs of strains of *D. melanogaster* at five stages of embryonic development. Figures are probability values. Scale in minutes from mean laying time. For description of stages, see text.

DISCUSSION

All of these data on developmental rates are fragmentary and preliminary. More observations and improvement in techniques would undoubtedly reduce confidence limits and increase the precision of probability inferences. But the data as they stand suggest interpretations and hypotheses of great interest which are worth elaboration and exploration as a basis for further thought and experiment.

It is clear that the three populations on which measurements have been made differ in developmental rates. But these differences are not simple in the sense that the rate of one population can be expressed in terms of the other times a constant. Instead, as development proceeds, relative rates seem to shift constantly, one population proceeding more rapidly up to a certain point, then slowing down, later speeding up again. Comparing Sy and SySP, for example, at stage I SySP appears to be ahead; at stage II Sy appears to have caught up; at stage III SySP is again out front; at stage IV the two are running neck and neck; but at stage V Sy has pulled away and is very significantly ahead. As between Sy and I_1, Sy is ahead at all stages from I through IV although the differences seem to be greater at I and IV than at II and III. After stage I, I_1 takes a spurt and narrows the gap, but after stage III it drops back again. By stage V, however, I_1 has pulled up and the two are running even.

Another way of visualizing these relationships would be to plot the times of arrival at successive stages of development by one population along the x-axis and the times of arrival at the same stages by another population along the y. If the two populations developed with identical rates throughout, the resulting curve would be a straight line with a slope of one. If the rates were different but had a constant ratio, the curve would still be a straight line but with a slope determined by the ratio. If, however, the ratio between the rates were shifting, we should obtain a line with breaks at the points of measurement, or, if we could make many measurements at very short intervals of time, we should probably obtain a smooth sinuous curve. The data definitely point in the direction of the latter situation.

Every cross-breeding population, which has existed long enough as a distinct entity in a given environment to approach equilibrium, has a characteristic distribution of phenotypes. It also has a characteristically integrated genetic system which means that the zygotes produced by the random combination of its gametes grow into adults showing the characteristic phenotypic distribution. Since the individual genotype actualizes the adult phenotype through the process of development, it would be surprising if every population did not have its proper developmental pattern. This is precisely what the data indicate with respect to developmental rates.

Within every living organism an extremely complex system of biochemical reactions is constantly going on. These reactions are interrelated through interactions or feedbacks which have the effect of maintaining the organism as a functioning unit. They hold its physiological states within limits characteristic of the population; they are responsible for its homeostasis. Dur-

ing development the biochemistry of the organism affects not merely the cyclical changes which enable it to stay alive, but in addition produces a series of directional changes which result in growth, differentiation and the construction of its morphology. Feedbacks direct these directional changes within limits which result in the adult phenotype; they are responsible for canalization or developmental homeostasis. In the Drosophila embryo a set of interacting biochemical systems determine the structural pattern of the larva and the potentialities of the anlagen of the imaginal discs which will eventuate in the characters of the adult. Thus, differences in the rates of different interacting chemical systems must be responsible for the differences in morphology, pigmentation and physiology which will characterize the adult flies. Differences in rates of embryonic development must mirror differences in the rates of the chemical reactions directing development. Thus, when we observe different patterns of developmental rates in two populations, we are seeing one kind of evidence of the different processes at work building the different characteristic phenotypic distribution.

Kacsar (Waddington, 1958 Appendix) has outlined a series of theoretical models in chemical kinetics based on open chemical systems approaching a steady state. These are very relevant to our data on developmental rates. His models indicate that differences in catalytic rates (different enzymes) will result in different ratios of components when the steady state is reached, but also that they will produce a different developmental pattern between the initial and the steady state. Depending on their position in the chemical system, changes in catalytic rates may have their effects partially or completely buffered out by the time the steady state is reached, but their effects will nevertheless be observable as differences in the rate at which the steady state is approached. Of course, not merely catalytic rates but other factors such as differences in temperature, in specificity of components or in their relative concentration have an influence on the steady state, on the approach to it or on both. But these complications do not make conceptual analysis impossible.

If we view the adult phenotype as the steady state, two different populations may achieve it by different chemical recipes in which case their developmental patterns will be different. Actually, of course, two populations do not achieve precisely the same steady state, for they always have differences in the means of some of their morphological, physiological and behavioral characters and in the distributions around these means. Nor do all the individuals within a population achieve the same steady state, for there are individual differences in morphology, physiology and behavior: the population is characterized by a set of distributions. As would therefore be expected, there is a temporal distribution of individuals within a population at any given developmental stage.

If two populations are approaching the steady state by way of different developmental patterns, one would suspect that they might exhibit morphological differences at some corresponding intermediate stage. Evidence to this effect has come to light in the case of Sy and SySP. Measurements

show that in a sample of blastulae of Sy the mean volume of the peripheral cells constitutes a significantly smaller proportion of the total volume of the embryo than they do in blastulae of SySP. The difference is sufficiently great to make it possible to distinguish samples of 15 or 20 from each population at a magnification of $\times 20$ even though some individuals overlap. Just how this difference is related to the differing rates of development is unknown, but it indicates that populations have characteristic patterns of development in morphology as well as on a time scale.

All the evidence indicates that different populations possess individual developmental patterns which can be investigated and described statistically. In these patterns biochemical, embryological and population phenomena are all integrated in a single matrix. Further investigation along these lines may very well increase our understanding of all the processes involved.

SUMMARY

In an effort to find common ground between the fields of population and developmental genetics, techniques have been worked out for observing and measuring some embryonic characters in *Drosophila melanogaster*. Measurements made on developmental rates in three strains show that each has its own developmental pattern. These characteristic patterns of development are evidence of the way in which the genetic system of a population produces the array of adult phenotypes characteristic of the population by effecting its own set of biochemical and embryonic events.

LITERATURE CITED

Dobzhansky, Th., 1950, Genetics of natural populations XIX. Origin of heterosis through natural selection in populations of *D. pseudoobscura*. Genetics 35: 288–302.

Dobzhansky, Th., and Bruce Wallace, 1953, The genetics of homeostasis in Drosophila. Proc. Nat. Acad. Sci. 39: 162–171.

Finney, D. J., 1952, Probit analysis. Second edition. Cambridge University Press.

King, James C., and Lauritz Sømme, 1958, Chromosomal analyses of the genetic factors for resistance to DDT in two resistant lines of *Drosophila melanogaster*. Genetics 43: 577–593.

Lerner, I. M., 1954, Genetic homeostasis. John Wiley and Sons, Inc.

Vetukhiv, M., 1956, Fecundity of hybrids between geographic populations of *Drosophila pseudoobscura*. Evolution 10: 139–146.

Waddington, C. H., 1958, The strategy of the genes. Macmillan Co.

Wallace, Bruce, and James C. King, 1951, Genetic changes in populations under irradiation. Amer. Nat. 85: 209–222.

1952, A genetic analysis of the adaptive values of populations. Proc. Nat. Acad. Sci. 38: 706–715.

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NEW SPECIES AND FORMS OF CEREALS DERIVED FROM HYBRIDIZATION BETWEEN WHEAT AND COUCH GRASS

N. V. TSITSIN AND V. F. LUBIMOVA

Principal Botanical Garden, Moscow, U.S.S.R.

Remote interspecific and intergeneric hybridization is one of the most important factors in the natural evolution of the organic world and a powerful method in creating new varieties and species of cultivated plants and breeds of domestic animals.

Hybridization between cultivated cereals and wild grass species is one of the leading ways in which this method is elaborated and used.

Our studies have established that all species of wheat cross relatively well with four species of genus Agropyron: *A. glaucum* Desf. (*A. intermedium* (Host) P.B., (2n = 42), *A. elongatum* (Host) P.B., (2n = 70), *A. junceum* (L.) P.B., (2n = 42), *A. trichophorum* (Link) Right (2n = 32) and one of the varieties (Siberian) of *A. repens* (L.) P.B., (2n = 42).

But not all of the foregoing species of couch-grass yield valuable hybrids. The hybrids with the greatest practical value are produced by crossing *Tr. vulgare* and *Tr. durum* with *A. glaucum* and *A. elongatum*. Both these species of couch-grass are quite polymorphic, especially *A. glaucum*. Special studies of a large collection of couch-grass gathered from different habitats have shown that many forms yield hybrids differing sharply in biological and morphological characters. Consequently, the couch-grass parent had to be selected according to its degree of perennial growth, winter or spring development, the biochemical composition of the grain, especially its protein content, and a number of other characters.

AGRONOMIC CHARACTERISTICS OF THE HYBRIDS

As a result of crossing wheat and couch-grass we have now produced not only new winter and spring varieties of common wheat (*T. aestivum*) but also new species of Triticum. One of these is the perennial wheat *Triticum agropyrotriticum perenne* (Cicin) and various forms of forage wheat *Triticum-agropyrotriticum submittans* (Cicin).

Triticum agropyrotriticum is a new 56-chromosome species of wheat differing from all other species not only in the number of its chromosomes, but also in more important characteristics such as the structure of the ear and the life cycle. The plants of this species do not end their cycle of development after the maturation of the grain, but yield new shoots which may produce a new grain crop under the conditions of the southern areas of our country or a good hay crop in the middle zone of the Soviet Union.

Triticum agropyrotriticum has a large number of varieties and forms differing from each other in structure, coloring, development of awns (beards), and pubescence. Characteristic of all the forms of this species is the rela-

tively loose structure of the ear, which somewhat resembles the speltoid forms of wheat but differs from them in a number of small characters. The grain of the fodder wheats is relatively large, 1,000 grains weighing 28–35 and sometimes 40 grams. The grain is, as a rule, highly hyaloid, and has high milling and baking qualities.

The plants are immune to fungus and bacterial diseases and do not lodge. They form many tillers or shoots and have a highly developed root system.

TABLE 1

Characteristics of a perennial wheat derived from hybridization between *Triticum vulgare* and *Agropyron glaucum*

Properties and characters	*Tr. vulgare ferrugineum* 2453	Perennial Wheat M2	*A. glaucum*
Cycle of development	Annual	Perennial (2–3 years)	Perennial 15 years and more
Character of pollination	Self-pollinating	Facultative Self-pollination	Cross-pollinating
Immunity to fungus diseases	Susceptible	Immune	Immune
Lodging	Present	Absent	Absent
Post-harvest growth	None	Average	Intense
Root system	Weak	Strong	Very strong
Process of maturation	Bottom upward	Top downward	Top downward
Number of stems per plant	5–10	20–25	50–100
Ear	Dense	Moderately lax	Very lax
Largest number of spikelets per ear	25	35	20
Largest number of flowers per spikelet	5	9	11, 0
Grain	Farinaceous	Hyaloid	Moderately hyaloid
Amount of protein in grain	15%	25%	20%
Number of chromosomes in somatic cell	2n = 42	2n = 56	2n = 42
Absolute weight (weight of 1,000 grains)	28–30 g.	30–33 g.	6 g.

To characterize the representatives of *Tr. agropyrotriticum*, table 1 shows data on the perennial wheat M 2 compared with the winter wheat *ferrugineum* 2453 and *A. glaucum*.

The perennial and forage wheats make possible a new use of the oldest cultivated crop, wheat, not only for grain with a high protein content, but also for green fodder or hay with a protein content equal to that in the grain of ordinary soft wheat.

By crossing varieties of winter wheat containing an average of 15 per cent protein in the grain, which is the highest content available, with individually selected clones of *A. glaucum* containing up to 30 per cent protein in the grain, we obtained perennial and forage forms of wheat with grains containing up to 25 per cent and hay with up to 15 per cent protein. It goes without saying that the nitrogen balance of the soil will, in one way or another, affect the amount of protein in the grain and in the hay of the hybrids, but the ratio between protein content of the hybrids and of ordinary wheat varieties remains more or less constant at different levels of soil fertility. The same picture is observed as regards the gluten content in the grain. It will be noted that wild species of Agropyron and Elymus contain large amounts of gluten. Thus, in Agropyron it ranges from zero to 70 per cent, and in Elymus—from 50 to 70 per cent, etc.

The forms of winter and spring wheat-couch-grass hybrids produced by crossing wheat with *A. glaucum* receive a high appraisal when tested under experimental and production conditions. Of these the winter forms 599, 186 and 1 are now sown to hundreds of thousands of hectares, while the spring wheats 56, 22850, 173, and others are undergoing state quality tests. They are noted for their high yield, they do not lodge, are immune to diseases and are highly plastic, owing to which they yield high crops under different climatic conditions.

THE OCCURRENCE OF NEW MORPHOLOGICAL CHARACTERISTICS OF THE FLORETS

In addition to valuable new agronomic characteristics, some plants of these hybrids exhibit new morphological characteristics not found in either of the parents. These arise as mutations and come to be inherited later. Remote hybridization acts as a kind of stimulus for the mutation process.

As is well known, every cereal plant possesses a flower comprising three stamens and one pistil, which at a later stage forms a caryopsis. Wheat-couch grass hybrids are of the same structure. But a few derivatives from such hybrids, including sometimes lines which are genetically constant, have flowers containing several pistils, rather than one, which later on give rise to double and triple kernels.

There are the following data in the literature on polycarpy in cereal crops:

Yudina and Uskova (1939) found in the "Mosdok" and Samurikum 329 winter barleys a large double caryopsis enveloped in the common flower hulls.

Rostovtseva (1951) mentioned the development of polycarpy in barley under the influence of certain environmental conditions.

Moh and Nilan in 1953 described a case of multiovary and many-seeded barley, which is a mutant induced by the atomic bomb explosion irradiation in Bikini in 1946.

Sadykhov in 1956 noted cases of double and triple kernels forming in wheat under the conditions of Azerbaijan.

The first information on the polystigmous flowers in wheat-couch-grass hybrids was reported by us in 1951.

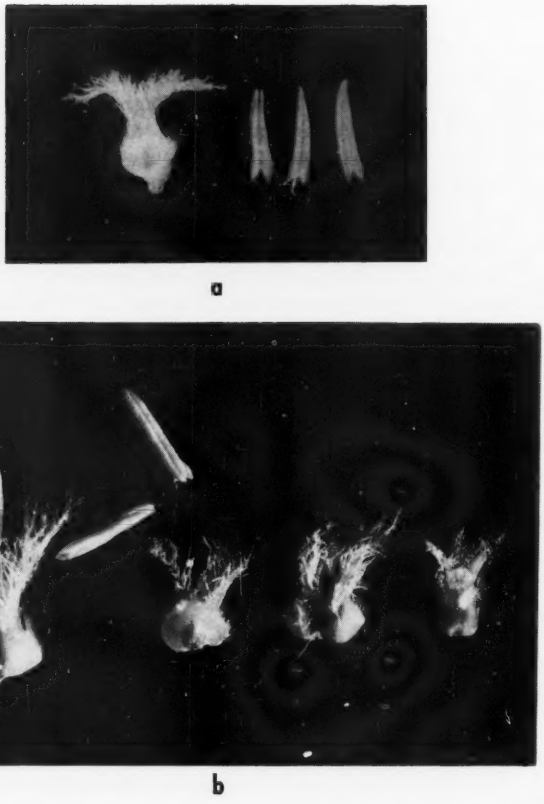

FIGURE 1. The generative parts of the perennial wheat-M2 flowers: (a) those of the normal flower with one pistil and three stamens; (b) those from a polystigmous flower with several pistils and three stamens.

In relation to the formation of polystigmous flowers, of interest is the perennial wheat-M2 developed by Academician N. V. Tsitsin through the hybridization of Saratovskaya 329 winter wheat × *Agropyron glaucum* Desf, with the first generation pollinated with the rye-wheat hybrid 46/131.

As we have found, this perennial wheat, which rather fully combines the traits of wheat and couch-grass and represents a variety of completely new-56 chromosomal species—*Triticum agropyrotriticum perenne* (Cicin)—appeared to have a few plants displaying individual flowers with two to three and more pistils with three stamens present.

Figure 1 shows the generative parts of the flowers from the perennial M2 wheat:

(a) those of the normal flower;

(b) those belonging to the polystigmous flower with several pistils and three stamens.

Later on these flowers produce double and triple kernels.

FIGURE 2. (a) The superior part of the perennial wheat-M2 head with double and triple kernels; (b) -M2 double and triple kernels set against the normal kernel of this wheat.

FIGURE 3. The generative parts of the flower in the F_1 M2 terats × *A. glaucum.*

Figure 2a represents the superior part of the head from the perennial wheat M2, where double and triple kernels are visible, while figure 2b shows these kernels in comparison with the normal kernel of this wheat. The cytological analysis of plants which developed from the double or triple kernels disclosed the fact that all of them had the complete diploid complement of chromosomes equal to 2n = 56.

Almost at the same time when polystigmous flowers were found a discovery was made of hybrids with teratological changes of another sort. The stamens in the flowers of these hybrids have been transformed, to a certain extent, into pistils or stamen-pistils, that is, formations combining the elements of gynoecium and androecium.

Figure 3 represents the generative parts of one of such flower which formed stamen-pistils instead of stamens. The degree of transformation in stamen-pistils can vary considerably.

Figure 4 shows the principal normal pistil and stamen-pistils from several flowers in various degrees of conversion.

All plants with these teratological changes belong to a first-generation hybrid obtained as a result of crossing the perennial wheat-M2 with couch grasses; *A. glaucum* Desf. or *A. elongatum* (Host) P.B., or with the F_1 hybrid couch grass (*A. glaucum* × *A. elongatum*).

Further crosses between the perennial wheat-M2 and the same varieties of couch-grass yielded hybrids showing the like teratological changes, but by far the greater number of these hybrids have the normal floral structure. The ratio of the plants with teratological changes to the over-all number of the first generation hybrid plants constitutes 5.7 per cent. The crossing of perennial wheat-M2 with the winter wheat varieties *Triticum vulgare* produced no teratological forms.

Up to the present time we have investigated more than 50 plants having teratological changes of a similar nature. It should be noted that different teratological plants, although of the same origin, have differing degrees and character of transformation. In some plants all the stamens have been completely transformed into pistils, while others have only individual stamens

transformed. Occasional plants can exhibit the formation of three stamen-pistils in the presence of three normal-structure pistils in the same flowers.

All these facts indicate that there is a segregation occurring in the perennial wheat-M 2, constant in all its principal characters, as regards the formation of polystigmous flowers and inheritance of the pistil transformation into stamen-pistils.

The degree and nature of the teratological changes are retained during the whole life time of the plants. They are also maintained in clonal divisions, but vary with growing conditions.

The anatomical structure of the stamen-pistils, which were formed from the stamens, is different depending on the rate of change. In almost unaltered stamens, there are only stigma elements in the superior part, and normal pollen sacs in the inferior part. Stamens, that suffered a greater alteration towards pistils, form even an ovary and ovules. It should be pointed out that in most cases one stamen produces two intergrown pistils. These pistils that have grown together, have polylobed and often irregularly-shaped stigmata (figure 4).

A transverse section through such double pistils formed from one stamen shows two intergrown ovaries and two ovules. The development of two ingrown pistils and one stamen provides new data for considering the origin of the pistils and stamens.

The first generation of hybrids from similar crossings is sterile and, as a rule, their anthers are not dehiscent. The second generation can be obtained through back-cross with wheat or wheat-couch-grass hybrids of the elder generations.

FIGURE 4. Rate of transformation of stamens into pistils.

The sterility of hybrids with teratological changes is many times greater. Numerous pollinations of them with wheat pollen and with fertile wheat-couch grass hybrids yield usually no result.

Nevertheless we have succeeded in producing F_2 plants with teratological changes. As distinct from F_1, the F_2 rate of change was lower and anthers were dehiscent. Of great interest in this respect are cases where the upper part of the anther contains elements of a pistil in the form of a stigma, while its lower part encloses a dehiscent anther with normal pollen.

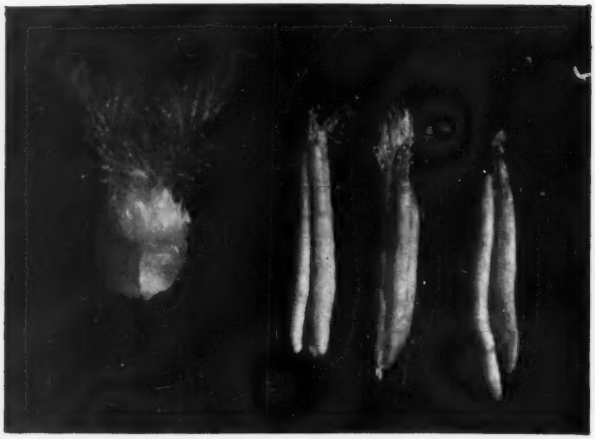

FIGURE 5. The degree of conversion from stamens into pistils in F_3.

Plants of the second generation are self-fertile but to an insignificant extent. In addition the seeds set from self-pollination under the conditions of spike isolation proved to be non-viable. The seeds resulting from free cross-pollination produced good F_3 plants. F_3 showed also segregation both in morphological and biological characters and as regards the anther structure.

Most F_3 plants had a reduced degree of change of stamens into pistils, as compared with F_1 and F_2 (figure 5), but at the same time these plants formed separate polystigmous flowers identical to those encountered in perennial wheat-M 2.

F_3 plants produced seeds on self-pollination. In this respect also of some interest is the progeny of one of the F_3 plants, having the maximum number of flowers that suffered alteration. Eight plants out of 11 had the changes once again, while the generative organs of the remaining 3 plants were of the normal structure.

F_4 plants showed an even lesser degree of transformation of stamens into pistils than F_3 plants, so that it revealed itself only in the appearance of hairs at the tips of stamens, which were similar to elements of the pistil

stigma. But the number of polystigmous flowers in F_4 considerably increased, which is apparent from table 2.

Thus, every successive generation decreases the rate of appearance of the double structure in stamens, that is, stamen-pistils, and increases the number of polystigmous flowers.

This phenomenon can be regarded as a process of normalization in the structure of floral organs, taking place with every generation which follows the F_1. The pistils formed from stamens develop the complete structure and perform the functions of a gynoecium, while the missing flower parts, that is, the androecium seem to be restored from new carpels.

TABLE 2

Degree of transformation of stamens into pistils and manifestation of polystigmous trait in F_1 to F_4 generations

Index for the rate of transformation of stamens into pistils and for the polystigmous trait	Generations			
	F_1	F_2	F_3	F_4
Rate of anther alteration on a 10– graduation scale	10	7	4	1
Number of changed flowers (in %)	100	93.5	73.5	18.7
Number of flowers having additional 2 to 3 pistils with the presence of 3 stamens (in %)	0	0.3	0.7	1.8

The formation of two intergrown pistils from one stamen in F_1 hybrids becomes transformed in later generations mainly in the direction of separating every pistil into an individual one, which leads to the appearance of polystigmous flowers. But a certain measure of connection is frequently retained at the base of the pistil. Not every pistil, however, undergoes a complete phase of development, some of them remain in an undeveloped stage.

In a few cases the pistils derived from one stamen are transformed in later generations into one pistil with a common ovary, but having two ovules. Later on some of them produce twin seeds, that is, seeds in the joint envelope, having two embryos. These seeds sprout into two shoots (see figure 6). The shoots can be relatively easily separated into two independent plants with the complete diploid complement of chromosomes in either of them.

It is interesting to note the correlation between the polystigmous feature in flowers and the general structure and biology of the perennial wheat-M2. During segregation in F_3 and F_4 it became evident that plants with a close likeness to the perennial wheat-M2 displayed a larger number of polystig-

FIGURE 6. Two shoots developing from twin seeds.

mous flowers, while the plants that differed considerably from the perennial wheat-M 2 had, as a general rule, the orthodox flower structure.

By virtue of systematic selection we could produce families of the perennial wheat-M 2, which produced a greater quantity of polystigmous flowers than the plants with this trait originally found.

The intensity of polystigmous manifestation in the perennial wheat-M 2 as well as the conversion of stamens into pistils in its hybrids may vary with environmental conditions.

The most extensive manifestation of these characters is due to the abundance of nutrients in the soil, high humidity, a shortened light day and a moderate temperature during the initial formation and differentiation of the spike.

As a rule the main shoots of the plant possess a larger number of polystigmous flowers and show an increased rate of conversion from stamens into pistils.

The described phenomenon provides an example of the emergence and inheritance of new structures. The same phenomena were observed in other

forms of the wheat-couch-grass hybrids, for instance, in the hybrid with a branching head and polystigmous flowers, but its polystigmous flowers have a different structure. They are formed as a result of the generative organs of two to three flowers being combined. The flowering glumes occurring between these flowers are formed as narrow and separated elements.

The study of the appearance and inheritance of new formations lays down the foundations for a deeper understanding of the problems of phylogeny and species formation and provides also an opportuntiy of creating new forms with useful traits.

LITERATURE CITED

Lubimova, V. F., 1952, Concerning the polyspermic quality in perennial wheat flowers. Bulletin Glavnogo Botanicheskogo Sada Akademii Nauk SSSR 13. (Russian)

Moh, C. C., and B. A. Nilan, 1953, Multiovary in barley. A mutant induced by atomic bomb irradiation. J. Hered. 44.

Rostovtseva, Z. P., 1951, Polycarpy and its development in annual cultivated cereals. Selektsiya i Semenovodstvo 10. (Russian)

Sadykov, A. M., 1956, Cases of double and triple wheat kernels. Botanicheskiy Zhurnal 10, No. 4. (Russian)

Tsitsin, N. V., 1958, Concerning the representatives of a new wheat species, *Triticum Agropyrotriticum* Perenne (Cicin). *In* Otdalennaya gibridizatsiya v semeistve zlakovykh [Remote hybridization in the family of cereals], USSR Academy of Sciences, Moscow. (Russian)

Yudina and Uskova, 1939, Polycarpy in barleys in wide row spaces. Yarovizatsiya 5-6 (26-27). (Russian)

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EXPECTATIONS ON RANDOM OCCURRENCE OF STRUCTURAL INTERCHANGES BETWEEN HOMOLOGOUS AND BETWEEN NON-HOMOLOGOUS CHROMOSOMES*

F. K. S. KOO

Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, Minnesota

Two of the reciprocal translocation types induced in seed by ionizing radiations, that persist through mitotic divisions, can be easily detected in microsporocytes. One of these recovered most commonly is the interchange between the non-homologous chromosomes which can be recognized at meiosis as a multiple association of chromosomes in the form of a ring or a chain. The other is the interchange between the opposite arms of the members of a pair of homologous chromosomes that results in formation of a pair of doughnut-shaped pseudo-isochromosomes. This latter type of interchange has been observed in plants grown from irradiated seed of barley (Caldecott and Smith, 1952; and Caldecott, 1955), maize (Morris, 1955), and oats (Koo, 1958). In *Avena strigosa* (2n = 14), the author found that the production of pseudo-isochromosome pairs, relative to the occurrence of rings (frequencies—three versus 20), seemed to be much higher than had been expected on a random basis. Caldecott (1955), likewise, was impressed by the relatively high incidence of this type of interchange and suggested two possible explanations: (1) the homologous chromosomes may be positionally related to one another in the cells of the dormant seed, thus facilitating the translocations between homologues, and (2) interchanges may take place between the break ends from the homologues more often than between those from the non-homologues.

In the literature, there has been contradictory evidence regarding the random occurrence of various types of chromosomal rearrangements produced by irradiation treatments. Rearrangements within a chromosome involving two breaks usually result in either inversions or deletions. Studies of Drosophila showed that inversion and deletion of the same length were produced about equally (Fano, 1941). Newcombe (1942) found in tulip and Tradescantia that rearrangement took place more often within an arm than between opposite arms of a chromosome, and likewise within a chromosome than between chromosomes. This is in agreement with Catcheside (1938), and Bauer, Demerec, and Kaufmann (1938) who observed that in Drosophila paracentric inversion occurred more frequently than pericentric inversion and translocation. In studies of inversions in the x-chromosome versus translocations between x-chromosome and autosomes in Drosophila, Kaufmann

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(1946) found that inversions were about two and a half times as frequent as expected on the random basis. The disparity between intra- and interchromosomal rearrangements becomes even more pronounced when females are irradiated. Glass (1940) produced only inversions but no translocations in the x-rayed Drosophila oöcytes. Similar results were reported by Crouse (1950) in Sciara. However, Catcheside, Lea, and Thoday (1946), in studies of irradiated Tradescantia microspores, found that the rearrangement frequency within an arm was significantly less than that between opposite arms of a chromosome at 1° and 20°C, but not significantly different at 30°C. They also reported that at lower temperatures there was some preference for exchange between breaks in the same chromosome as compared with exchange between breaks in different chromosomes, but that the rearrangements became more nearly random as the temperature was raised. In comparing the different rearrangement types that resulted from two breaks in different chromosomes, Catcheside, Lea, and Thoday (1946) found that both symmetrical and asymmetrical rearrangements in Tradescantia occurred with about equal frequencies. This is in agreement with the results of Newcombe (1942), but in disagreement with that of Sax (1940) who observed an excess of dicentrics over exchanges.

The availability of information on the structural interchanges between homologous and between non-homologous chromosomes appears to provide an excellent opportunity to re-examine the problem of random recombination if the breakages occur at random. These two types of rearrangements are highly suitable for the comparative study of their frequency of occurrence because both types are formed in the same manner, namely by a recombination involving two breaks in different chromosomes. If the breakage and recombination occur at random, then a definite proportion for each type is expected in a given organism. This paper presents (1) the assumptions and basis for calculating the expected frequencies of the two types of interchanges if the breakages and recombinations are random, (2) comparison of actual frequencies with the theoretical, and (3) discussion of the results.

In the development of a generalized formula for deriving the theoretical expectations of the two types of interchanges, the following assumptions were made regarding chromosome breakages: (1) each chromosome has two arms, (2) all arms have equal breakability with any given level of irradiation, (3) two breaks, each in a different chromosome, must occur at the same time or within a time limitation to allow formation of either of the two types of interchanges, (4) the two breaks will involve two homologous or two non-homologous chromosomes at random, and (5) if two homologous chromosomes are involved, only the cases in which the two breaks are in non-homologous arms may result in the pseudo-isochromosome type of interchange.

With regard to behavior of the break ends, the following facts and assumptions were used: (1) the types of interchanges observed result only when the break ends rejoin in new combinations, (2) only the recombinations that produce two monocentric interchanged chromosomes will result in the two interchange types being studied, and (3) any two arms of different chromosomes,

homologous or non-homologous, have an equal chance of being so arranged spatially in the nucleus as to permit exchange of segments when breaks occur.

The formation of all possible breakage combinations can be illustrated with two pairs of chromosomes as follows:

On these two pairs a total of 24 possible breakage combinations is expected:

Group A

1-3, 1-4, 2-3, 2-4
5-7, 5-8, 6-7, 6-8

and

Group B

1-5, 1-6, 2-5, 2-6
1-7, 1-8, 2-7, 2-8
3-5, 3-6, 4-5, 4-6
3-7, 3-8, 4-7, 4-8

The eight combinations in Group A involve homologues and the 16 in Group B involve non-homologues. Four combinations are possible from any two chromosomes. Mathematically, this may be expressed as $\binom{M}{m}\binom{M}{m} = 4$ where $M = 2$, that is, the number of possible breaks which can be produced in each chromosome with one break in each arm, and $m = 1$, that is, the number of breaks from each chromosome involved in the formation of each recombination.

For an organism with N pairs of chromosomes, the total number of possible breakage combinations is $4\binom{2N}{2}$.

Although four breakage combinations are possible in each pair of homologues, only two of them would yield pseudo-isochromosomes, the other two being interchanges between the homologous arms. Therefore, the readily detectable interchanges involving a pair of homologues, that is, those resulting in pseudo-isochromosomes, would be only one-half of the total or two. In an organism with N pairs of chromosomes, the expected frequency of interchanges producing pseudo-isochromosomes is 2N.

Since four breakage combinations are possible from any two non-homologues, then in two pairs of chromosomes four times as many combinations are expected. This can be expressed as $4\binom{M}{m}\binom{M}{m} = 16$. In this case, all 16 possible breakage combinations are capable of producing interchanges which

would lead to the formation of rings of fours. For an organism having N pairs of chromosomes, the expected frequency of interchanges between non-homologues is $16\binom{N}{2}$.

Therefore, the expected ratio for the random occurrence of rings and pseudo-isochromosomes in an organism with N pairs of chromosomes is $16\binom{N}{2} : 2N$. By dividing both terms in the ratio by 2N, this gives

$$\begin{aligned} 16\binom{N}{2} / 2N : 2N / 2N &= 8\binom{N}{2} / N : 1 \\ &= \frac{8}{N} \times \frac{N(N-1)}{2!} : 1 \\ &= 4(N-1) : 1 \end{aligned}$$

With this generalized expression, the theoretical ratio for the two interchange types to occur in any given species can be readily obtained by simply substituting the number of chromosome pairs of that species instead of N in the ratio. For barley and *Avena strigosa*, both of which have seven pairs of chromosomes, the expected ratio is $4(N-1) : 1 = 24 : 1$ and for maize which has 10 chromosome pairs the ratio expected is 36 : 1.

In this paper only the frequency data amassed by Caldecott and Smith, and Caldecott for barley and by Morris for corn will be examined by the χ^2 test for goodness of fit to their respective theoretical ratios. A limited amount of information on *Avena strigosa* does not allow such analysis. Due to the fact that the cells from which these data were obtained included those with multiple and complex as well as simple interchanges, each series was treated as follows:

1. Frequencies of interchanges were determined only from the cells with one ring of four or a pair of pseudo-isochromosomes, each of which was counted as one.
2. Frequencies of interchanges were determined from all cells, including those with multiple and complex interchanges. A ring of four or a pair of pseudo-isochromosomes was counted as one. A ring of six was counted as 1.5 since the least number of breaks required for such a rearrangement is three compared with two for the ring of four or the pair of pseudo-isochromosomes. On the same basis a ring of eight or ten was counted as two or 2.5 interchanges, respectively.
3. Frequencies were determined as above except that a ring of six, eight, or ten was counted as two, three, or four reciprocal interchanges, respectively, on the assumption that four, six, or eight breaks, respectively, were involved in the formation of each of these larger rings. This method of counting is favored by some workers over the second method.

Frequencies determined by these three methods are available for all series except one for which information was not available for calculation by method 1 (see table 1).

TABLE 1

Frequencies of interchanges between non-homologues and between homologues produced by various ionizing radiations in barley and corn and the χ^2 tests for goodness of fit of observed frequencies to theoretical ratios (24 : 1 for barley and 36 : 1 for corn)

Material	Series	Radiation	Method of frequency counting	No. interchanges between non-homologues	No. interchanges between homologues	χ^2	P	Source of frequency data
Barley	1	X-rays (34 KVP, 27 ma., unfiltered)	1	...	...	...	...	Caldecott and Smith 1952
			2	466.5	29	4.429	.05–.02	
			3	477	29	3.949	.05–.02	
	2	X-rays (250 KVP, 30 ma., 1 mm. Al filter)	1	252	12	0.205	.70–.50	Caldecott 1955
			2	313	12	0.080	.80–.70	
			3	323	12	0.152	.70–.50	
	3	X-rays (250 KVP, 30 ma., 1 mm. Al filter)	1	201	7	0.218	.70–.50	Caldecott unpublished
			2	265.5	8.5	0.575	.50–.30	
			3	275	8.5	0.741	.50–.30	
	4	Gamma rays (Co^{60} source)	1	205	7	0.269	.70–.50	Caldecott unpublished
			2	238	12	0.417	.70–.50	
			3	244	12	0.315	.70–.50	
	5	Thermal neutrons (thermal column of nuclear reactor)	1	486	21	0.027	.90–.80	Caldecott 1955
			2	758	27	0.642	.50–.30	
			3	811	27	1.321	.30–.20	
	6	Fast neutrons (U^{235} fission)	1	177	18	13.894	<.01	Caldecott unpublished
			2	278	23.5	11.304	<.01	
			3	296	23.5	9.367	<.01	
Corn	7	X-rays (250 KVP, 30 ma., 1 mm. Al filter)	1	98	4	0.576	.50–.30	Morris 1955 and unpublished
			2	156	5.5	0.303	.70–.50	
			3	166	5.5	0.166	.70–.50	
	8	Thermal neutrons (thermal column of nuclear reactor)	1	251	5	0.547	.50–.30	Morris 1955 and unpublished
			2	466.5	12	0.069	.80–.70	
			3	494	12	0.211	.70–.50	

The P values obtained from the χ^2 test for each series of data indicate that the observed frequencies showed a satisfactory fit to the theoretical expectations for the barley series 2, 3, 4, and 5, and for the corn series 7 and 8. The P values for series 1 are between .05–.02, indicating a poor fit; and that for series 6 are much less than .01, suggesting a significant deviation from the expectation. These deviations observed in series 1 and 6 are in the direction of excess of pseudo-isochromosomes.

Despite these two exceptional cases the majority of the evidence in this study indicates that the interchanges between chromosomes, homologous or non-homologous, occurred at random. It is difficult to understand why an excess of pseudo-isochromosomes was observed in series 1 and 6 in view of the fact that the radiations of both low and high ion density employed in other series did produce the two interchange types at random. Apparently such exceptional cases can hardly be interpreted on the basis of physical differences of the radiations.

Since the theoretical expectations were derived from considering only two breaks in a nucleus, it would be of interest to compare the results obtained from the analyses with and without the multiple and complex interchanges included. It appears that there were no appreciable differences between the results of the three methods of calculating the interchange frequencies. In fact, differences would hardly have been expected because of the relatively low frequency of multiple and complex interchanges.

SUMMARY

On the assumption that the breakages and recombinations occur at random, a generalized expression for calculating the expected ratio of rings and pseudo-isochromosomes in any given organism was derived. Results from the comparisons of observed data for barley and maize with the theoretical except in two cases indicate that the interchanges between chromosomes, homologous or non-homologous, were random.

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LITERATURE CITED

Bauer, H., M. Demerec and B. P. Kaufmann, 1938, X-ray induced chromosomal alterations in *Drosophila melanogaster*. Genetics 23: 610–630.

Caldecott, R. S., 1955, The effects of x-rays, 2-Mev electrons, thermal neutrons, and fast neutrons on dormant seeds of barley. Ann. New York Acad. Sci. 59: 514–535.

Caldecott, R. S., and L. Smith, 1952, A study of x-ray-induced chromosomal aberrations in barley. Cytologia 17: 224–242.

Catcheside, D. G., 1938, The effect of x-ray dosage upon the frequency of induced structural changes in the chromosomes of *Drosophila melanogaster.* J. Genetics 36: 307-320.

Catcheside, D. G., D. E. Lea and J. M. Thoday, 1946, Types of chromosome structural change induced by the irradiation of Tradescantia microspores. J. Genetics 47: 113-136.

Crouse, H. V., 1950, The differential response of male and female germ cells of *Sciara coprophila* (Diptera) to irradiation. Amer. Nat. 84: 195-202.

Fano, U., 1941, On the analysis and interpretation of chromosomal changes in Drosophila. Cold Spring Harbor Symp. Quant. Biol. 9: 113-121.

Glass, H. B., 1940, Differential susceptibility of the sexes of Drosophila to the effect of x-rays in producing chromosome aberrations. Genetics 25: 117.

Kaufmann, B. P., 1946, Organization of the chromosome. I. Break distribution and chromosome recombination in *Drosophila melanogaster.* J. Exp. Zool. 102: 293-320.

Koo, F. K. S., 1958, Pseudo-isochromosomes produced in *Avena strigosa* Schreb. by ionizing radiations. Cytologia 23: 109-111.

Morris, Rosalind, 1955, Induced reciprocal translocations involving homologous chromosomes in maize. Amer. J. Bot. 42: 546-550.

Newcombe, H. B., 1942, The action of x-rays on the cell. I. The chromosome variable. J. Genetics 43: 145-171.

Sax, K., 1940, An analysis of x-ray induced chromosomal aberrations in Tradescantia. Genetics 25: 41-68.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

A PARTIAL DOMINANT FOR SUPPRESSION OF COLOR IN THE SYRIAN HAMSTER, *Cricetus Mesocricetus auratus*

In the last few years there have been several reports of a blind, pink-eared albino hamster, recently described by Knapp and Polivanov (1958). In contrast to the dark eared albino (designated $c^d c^d$ by Robinson) in which faint grey pigmentation of the ears appears at about four weeks and develops fully to a deep grey or black by maturity, pigmentation is completely suppressed in the blind albino. In addition these animals demonstrate an incomplete optic development, while the dark-eared albino's eyes function normally. Knapp and Polivanov noted anophthalmia in all their animals, but microphthalmic albinos have been reported by other breeders.

Albert Marsh reported (unpublished observations, 1955) that blind whites had appeared several years previously in Wisconsin, and had been bred to several hundred specimens. Bred to normal goldens, a golden F_1 resulted, and the F_2 consisted of golden and blind whites. Bred to the dark-eared albino, a golden F_1 was again produced, and the F_2 was made up of golden, dark-eared albino, and blind whites. Marsh, as well as Knapp and Polivanov, came to the conclusion that the factors for complete albinism and blindness could not be separated.

Recently we obtained an extremely white panda, or piebald, (*ss*) male hamster with ruby eyes. (This dilution of eye color is often produced in the panda when the pattern of white spotting passes over the eye.) This male, bred to a normal female carrying a panda factor, produced an F_1 of 16, of which nine survived. Of these three were normals, while the other six were distinguished by distinct ruby eyes and a white ventrum (WV) (in contrast to the usual ventral coloring of light grey underfur with pale cream tips). At maturity a few small clumps of white hair were discernible in the dorsal area of some WV. None exhibited the white blaze normally associated with panda. The lack of pandas may be attributed to their typical lower viability and inability to cope with competition of hardier littermates.

A pair of WV were bred, resulting in the appearance of two blind pink-eared albinos (BA), along with five WV and two normals. At least one BA has occurred in all subsequent F_2 (WV × WV) litters. These albinos differ from those of Knapp and Polivanov only in the matter of eye development, being characterized by microphthalmia rather than anophthalmia. Size of the eyeball at 19 days is one-half to one-third that of a normal littermate. In addition, the eyeball may be either opaque or clear. It is of interest to note

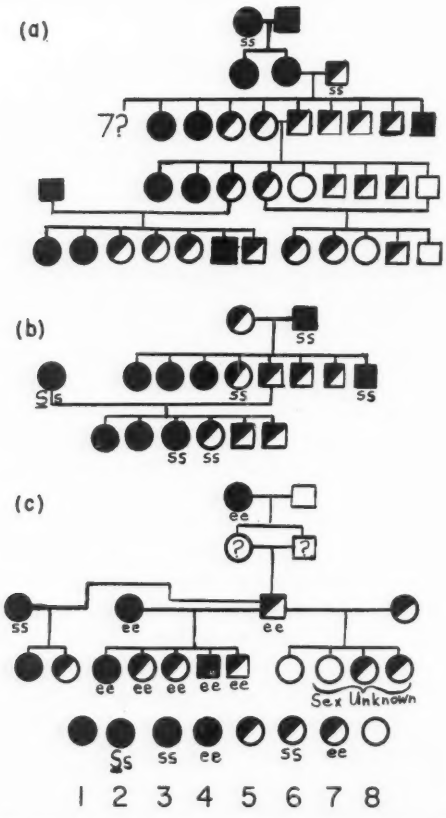

FIGURE 1. Pedigrees involving the partially dominant gene for white: (a) Production of microphthalmic albinos; (b) Production of whiter pandas; (c) Production or ruby-eyed whites. Code: circles are females, squares males. Designations for mutant factors are: s, panda; e, cream; Wh, white. Phenotypes and probable corresponding genotypes are: (1) normal, *S-E-wbwb*; (2) normal carrying panda, *SsE-wbwb*; (3) panda, *ssE-wbwb*; (4) cream, *S-eewbwb*; (5) white ventrum, *S-E-Wbwb*; (6) whiter panda, *ss E-Wbwb*; (7) ruby-eyed white, *S-eeWbwb*; (8) microphthalmic albino (or BA), *WbWb*.

that a given animal may exhibit one clear and one opaque eye. The eyelids apparently remain closed at all times, although they may be opened manually.

A total of 76 F_2 animals were born, with 16 BA (21 per cent), 45 WV (59 per cent), and 15 normals (20 per cent). WV × normals produced 84 WV (55 per cent) and 69 normal (45 per cent). Personal communications from J. Draheim and R. Warren also give approximately the 1-2-1 ratio in the F_2 series, and 11 BA (26 per cent) out of a total of 42 young from a mating of WV × BA. All of these results are in good approximation to the expected ratios for a partial dominant expressed phenotypically by ruby eye and white ventrum in the heterozygous, and by microphthalmic albinism in the homozygous, condition.

The very white panda male was unavailable for further breeding. The F_1 WV female was bred to a normal panda, producing three normal golden, three WV, one normal panda, and one very white panda. Further WV (split for panda) × panda matings gave nine normal, 11 WV, four panda, and four whiter panda; or 13 non-WV : 15 WV, and 20 non-panda : eight panda. (Expected ratios 1 : 1. Panda ratios usually are deficient by approximately 50 per cent due to lower viability.)

The WV (carrying panda) were bred to normals carrying panda. From these, 16 normal, 19 WV, five panda and seven whiter panda resulted. (21 non-WV : 26 WV; 35 non-panda : 12 panda. Expected ratios 1 : 1 and 3 : 1 respectively. We have found less deficiency of pandas from hybrid parents, apparently due to heterosis.) An independent segregation of the WV and panda genes is apparent, since our panda stock has been uniformly much browner than these whiter pandas. We have assumed that the whiter pandas carry the WV gene, and test breeding to check this is in progress. The 1 : 1 ratio was also observed when WV was bred to normal carrying no panda factor.

Robinson has suggested an analogy with a known mouse factor *Wb*, which produces a "mock albino" with complete suppression of pigment and a microphthalmia when *WbWb*; and a variable ruby eye with some reduction in yellow and possibly some spotting, when *Wbwb*.

Adopting the designation *Wb*, the probable corresponding phenotypes and genotypes would be as follows:

normal	*wbwb*	panda	*sswbwb*
WV	*Wbwb*	whiter panda	*ssWbwb*
BA	*WbWb*	English cream	*eewbwb*
		Ruby-eyed white	*eeWbwb*

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LITERATURE CITED

Draheim, J., and R. Warren, Personal communications.

Knapp, B., and S. Polivanov, 1958, Anophthalmic albino: a new mutation in the Syrian hamster, *Cricetus* (*Mesocricetus*) *Auratus*. Amer. Nat. 92: 317–318.

Marsh, A., 1955, Unpublished observations.

Robinson, R., 1957, Partial albinism in the Syrian hamster. Nature 180: 443–444.

Personal communication.

MARGARET E. BEHER
WILLIAM T. BEHER*

EDSEL B. FORD INSTITUTE
FOR MEDICAL RESEARCH*
HENRY FORD HOSPITAL
DETROIT 2, MICHIGAN

February 9, 1959

BLYTH, DARWIN, AND NATURAL SELECTION

Professor Loren C. Eiseley, the author of an excellent recent book on the history of evolutionism (Eiseley, 1958), has just published a study (Eiseley, 1959) of the forgotten work of an English naturalist, Edward Blyth (1810-1873). The matter is of truly exceptional interest, for Eiseley argues not only that the basic tenets of the theory of natural selection were stated by Blyth much earlier than by Darwin, but also that Darwin was familiar with Blyth's idea, made use of it in his theories, and yet failed to acknowledge his obligation to Blyth. It would seem that every biologist really owes it to himself to examine Eiseley's arguments, and to reach his own conclusion concerning the issue.

It must, I think, be conceded that Eiseley has made a most remarkable discovery. He has, with scrupulous care and patience, unearthed historical documents of absorbing interest. And, very properly, he has appended to his article the texts of Blyth's essays, originally published in The Magazine of Natural History, volumes 3 (1835), 9 (1836), and 1 (n.s., 1837), as well as the biographical memoir on Edward Blyth, published by A. Grote in the Journal of the Asiatic Society of Bengal (1875). A reader is thus enabled to delve into documentary evidence, without which Eiseley's conclusions would be well-nigh incredible. Eiseley believes that "... Blyth is more than a Darwinian precursor, that he is, instead, a direct intellectual forebearer in a phylogenetic line of descent. Edward Blyth, in the writer's estimation, belongs to the royal line. He is not an isolated accident. Instead, he is one of the forgotten parents of a great classic. But Darwin's shadow, grown to almost superhuman proportions, lies massive and dark across the early portion of the century."

It is not my purpose to summarize here either the works of Blyth or the arguments of Eiseley, for this would inevitably do injustice to both. It is impossible however to gainsay the conclusion of Eiseley that the fundamental premises of the theory of natural selection are contained in Blyth's essays. This indeed is important enough, and this is not a case of a modern biologist reading a modern meaning into ambiguous statements of an older author. It is astounding that the essays of Blyth remained unappreciated for more than 120 years. These essays were not published in an obscure provincial journal, like the work of Mendel was. The Magazine of Natural History was one of the major biological periodicals of the Blyth-Darwin age. Eiseley has used an almost detective-like ingenuity to prove that Darwin was familiar with the essays of Blyth. Why, then, did Darwin omit all mention of this fact? Why did he choose instead to say that the germ of the theory of natural selection was suggested to him by the reading of the work of Malthus?

We may never learn the answers. Blyth was not an evolutionist; he ascribed to natural selection the maintenance of the species boundaries, not the breaking of the species barrier. He did not use the words "natural selection." This does not however suffice for an explanation. Malthus was

not an evolutionist either; we know that there are stabilizing as well as dynamic forms of natural selection. Eiseley's opinion is as follows: "Charles Darwin, often depicted as a simple, forthright man, was in reality an enormously complex human being.... In this hundredth anniversary year since the publication of the *Origin*, the number of eulogies, addresses, and similar encomiums is burying even deeper the true story of the past. The flash of genius, the master stroke, arouse our pride in human achievement. Few of us want to learn that many less fortunate men toiled to erect the edifice later to be known as Darwinism. Fewer still will accept the fact that Darwin was a human man among men who yearned hungrily for the approbation of the world perhaps, as has been intimated by psychologists, as a compensation for the doubts of a domineering father. Yet it is this man, not the bearded idol, who is one of us if we would but see it."

Eiseley's opinion cannot be brushed aside, but the problem may be even more complex than he suggests. Precisely because Darwin was a "human man," should we not guard against the temptation of representing his thinking processes as wholly free of subconscious components which occur in other representatives of the species? Is the creative activity of even the greatest mind always following the canons of logic as strictly as an ex post facto analysis is liable to assume? Have not creative personalities, great as well as not so great ones, scientists as well as artists, testified again and again that ideas, insights, and generalizations came to them with dramatic suddenness, and often under singularly irrelevant and even incongruous circumstances? Yet another discoverer of the theory of natural selection, A. R. Wallace, arrived at this discovery during an access of malaria! Darwin's well-known story is that the similarity between Malthus's conception of the sociological processes and his own picture of the biological ones became clear to him while he was in a carriage on a country road. Assuming that the idea of natural selection was really suggested to Darwin by Blyth rather than by Malthus, does it follow that Darwin was dissembling his true sources? Is the origin of every idea which crosses our minds always clear to us? Nobody can claim such a perfection of memory and such an awareness of one's thinking processes. Probably everyone is familiar with the feeling that an idea which arises in one's mind, or a phrase which emerges from one's pen, have been met with somewhere, but one cannot recall just where or when. This feeling is sometimes justified but perhaps more often illusory. Might not even Darwin have been mistaken about the sources of some of his ideas?

The problem which the work of Eiseley forces us to face is in reality nothing less than the mystery of the creative process in the mind of a genius. Only rarely has an insight into this process been gained, and the picture which emerged proved far from simple. Lowes (1927) was able to trace perhaps more successfully than anyone else the history of the composition by Coleridge of some of his greatest peoms. And this was a history of "...the conscious seizing and directing to an end of suggestions which the unconscious operations have supplied.... Creative genius, in plainer terms,

works through processes which are common to our kind, but these processes are superlatively enhanced. The subliminal agencies are endowed with an extraordinary potency; the faculty which conceives and executes operates with sovereign power; and the two blend in untrammelled interplay." (This writer is thankful to Dr. Alfred Mirsky and Dr. Harold Simon for having called his attention to the book of Lowes.)

It is not illegitimate to compare the creative processes of a poet, Coleridge, with those of a scientist, Darwin. The work of a poet and of a scientist are, after all, not fundamentally different. Darwin did not necessarily have a much greater awareness of the soil in which his theories were growing than Coleridge of that which produced his poetry. Eiseley's remarkable discovery rescues from inexcusable oblivion the memory of a remarkable man, Blyth. But I find nothing in this discovery to pull "the bearded idol" down from his pedestal.

LITERATURE CITED

Eiseley, Loren C., 1958, Darwin's century. Doubleday & Company, Inc., New York.

1959, Charles Darwin, Edward Blyth, and the theory of natural selection. Proc. Amer. Philos. Soc. 103: 94–158.

Lowes, J. L., 1927, The road to Xanadu. Houghton Mifflin Company, Boston and New York.

THEODOSIUS DOBZHANSKY

DEPARTMENT OF ZOOLOGY
COLUMBIA UNIVERSITY
NEW YORK, NEW YORK
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HIGH FREQUENCY OF CHROMATID BREAKS IN TWO *in vitro* CELL POPULATIONS*

Spontaneous chromosome or chromatid breakages and reunions have been found in both *in situ* and *in vitro* cell populations, but the frequency is exceedingly low.

McCoy and his associates claimed that cells of Jensen rat sarcoma (McCoy et al., in press), like those of the Walker carcinosarcoma 256, require asparagine in the medium to grow in tissue culture. However, using his medium formula 5 a (McCoy et al., 1959) without asparagine, McCoy succeeded in establishing two mutant cell lines, JA-1 and JA-2, which did not require asparagine. At the tenth and eighth subculture in medium without asparagine, Strains JA-1 and JA-2 were shipped to this laboratory for cytological checkup. Two sets of colchicine-hypotonic-squash preparations were made. The modal chromosome numbers for both lines were registered at low 60's. However, like the populations of the Novikoff hepatoma cell lines (Hsu and Klatt, 1959), most cells in JA-1 and JA-2 displayed various combinations of their marker chromosomes so that the populations were highly polymorphic (detailed cytological data for Jensen sarcoma and its derivatives will be presented elsewhere).

FIGURE 1. Photomicrographs of chromosomes showing various configurations as results of chromatid breaks. Strain JA-2, Subculture 10, Colchicine-hypotonic-squash. Phase contrast.

An interesting phenomenon was observed in these populations, namely, there was an extremely high frequency of complex chromosomal configurations suggestive of chromatid exchanges. Figure 1 depicts a few such configurations. Chromatid exchanges may involve two, three or four chromosomes (figures 1 a–i). Figure 11 shows a distinct break on one chromatid,

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and figures 1 j and 1 k are examples of dicentric chromosomes, probable survivors of chromatid exchanges in the past mitotic generations. In a few cases, end-to-end attachment between sister chromatids were found. A survey of over 100 cells each disclosed that approximately 40 per cent of the cells contained one or more chromatid anomalies.

The original Jensen sarcoma cells grown in primary cultures containing asparagine did not show any such abnormality.

There is so far no explanation of this phenomenon of high frequency of chromatid exchanges in these cell populations, especially in the absence of chromosome breakages. The strains have been carried in routine manner without contact with any source of radiation and without even the use of trypsin when subcultures were made.

LITERATURE CITED

Hsu, T. C., and O. Klatt, 1959, Mammalian chromosomes *in vitro*. X. Heteroploid transformation in neoplastic cells. J. Nat. Cancer Inst. 22: 313–339.

McCoy, T. A., M. Maxwell and P. F. Kruse, 1959, Amino acid requirements of the Novikoff hepatoma *in vitro*. Proc. Soc. Exp. Biol. and Med. 100: 115–118.

McCoy, T. A., M. Maxwell, E. Irvine and A. C. Sartorelli, in press. Proc. Soc. Exp. Biol. and Med.

T. C. HSU
G. K. MANNA

SECTION OF CYTOLOGY
UNIVERSITY OF TEXAS M. D. ANDERSON
HOSPITAL AND TUMOR INSTITUTE
HOUSTON, TEXAS
March 18, 1959

Permanent address: Department of Zoology, Bangabasi College, Calcutta, India.